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Editor
HAMILTON B. G. ROBINSON

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EDITOR'S VIEWPOINT

One of the problems of dental clinicians and investigators has been to obtain useful radiographs of the temporomandibular joint. Craddock (p. 302) has investigated this problem and reports anatomic deviations of importance in about 30 per cent of patients. He observed well-delineated temporomandibular joint radiographs in about 70 per cent of more than 100 patients. A study was made of thirty healthy young male patients, free of joint symptoms, and from these norms were calculated. A second group of twenty completely edentulous adults was studied similarly. It is interesting that the norms of the latter did not differ essentially from those of the first group.

Yurkstas (p. 322) tested the effect of exercise (mastication) on biting forces of individual teeth in young adults and in children with orthodontic anomalies. In the young adults, the biting force was increased on the side preferred during experimentally supplemented mastication, although no change occurred on the opposite side. Among the children with occlusal disturbances, the added function increased bite force, especially in the first molar region. The author made no effort to determine whether increased biting force improves oral health, or whether it is undesirable.

Curby (p. 328) describes a simplification of the apparatus developed by Yurkstas and Manly for measurement of occlusal contact areas. The instrument, which is less expensive and is portable, offers assistance in evaluating food mastication efficiency.

Belting, Schour, Weinmann, and Shepro (p. 332) studied periodontal age changes in the molar region of two strains of rats. In the animals studied they concluded that the downgrowth of the epithelial attachment is related to age, not inflammation. They also found the increased distance, with age, between the alveolar crest and the cemento-enamel junction to be due to the eruption rate being faster than the rate of bone deposition at the alveolar crest. There were significant differences between the two strains, suggesting that there may be still more significant differences between these animals and man.

Mahler and Asgarzadeh (p. 354) found that dental plasters contract on setting and that the apparent outer dimensional increases must be caused by porosity. Despite the investigations that have been made on dental gypsum products, it is evident that more fundamental studies might lead to marked improvement in the product used in clinic and laboratory.

Salpeter (p. 362) attempted to reproduce, in vitro, the conditions under which cementum is deposited. Under the conditions of his study, he demonstrated the adverse influence of cholesterin on calcium precipitation and the beneficial influence of lecithin, vitamin D, and certain amino acids. It is suggested that high cholesterin levels in such diseases as diabetes and myxedema may be the cause of periodontosis.

A series of three papers by Knox and Still, and Knox (pp. 367, 374 and 379) report some observations on salivary constituents. The salivary mucoids were investigated first, and their fluctuation during the day points up the danger in attempting caries activity correlations with a single salivary analysis. Knox obtained partially purified salivary m u c i n a s e and, in vitro, found it inactivated at pH levels that might be present in areas of caries activity. Mucinase-producing b a c t e r i a (gram-negative rods) were found in saliva. This phase of the study indicates that carbohydrate can be produced in vivo from salivary mucoid. The enzyme system has been investigated and it is proposed that more than one enzyme is active.

Ogilvie (p. 386) studied the viscera of eleven rats maintained for 15 to 100 days in states of acute fluoride poisoning. The most dynamic effect was in the kidney, with lesser disturbance in the pancreas, thyroid,

and adrenal. Experimental investigation of the effects of fluorides on the health and growth of all body structures is of extreme importance in view of widespread fluoridation. Although caution must be taken less misinformed or uninformed individuals use data from studies of acute fluorosis as evidence of changes with very small quantities of fluorine, this danger does not justify suppression of open discussion. Information gained from these investigations may be used to guide necessary continuing studies in naturally or artificially fluoridated regions.

The function of Meckel's cartilage has been disputed, chiefly because the normal growth process in bone resorbs the evidence of the earlier pattern and because no fixed points are available for several measurements. The *ia* strain of rat does not have normal bone resorption, but its bone and cartilage are deposited in approximately normal fashion. Using this animal for detailed study during development, Bhaskar, Weinmann, and Schour (p. 398) have shown that Meckel's cartilage plays an important part in the growth of the mandibular process and mandible of the rat. Here an abnormal growth pattern has been utilized to explain normal development, and to settle a controversial point.

Kvam reports further on his study of the development of the fish. The present report (p. 411) is on dentin formation in Teleostei. The investigation included five of the bony fishes. The formation of dentin from collagenous fibrils was observed in these animals.

Investigations in the field of dentistry should not be limited by the use of tools with which the dentist is familiar through his usual dental education. Many new technics, available to scientists in general, are being utilized by dental investigators with effect. The x-ray diffraction technic has been used for investigation of dental tissues for over twenty-five years. It appears particularly adapted for study of normal and abnormal enamel, dentin, bone, and dental materials. Trautz, Klein, Fessenden, and Addelston (p. 420) interpreted x-ray diffractograms of enamel as showing enamel to be an apatite, fibrillar arrangement. They describe the application of the x-ray diffraction method.

Hurst, Nuckolls, and Conlon present a new technic for preparation of organic matrix of enamel (p. 432). This double embedding method employs agar as a support for the delicate organic gossamer.

Bender, Pressman, and Tashman (p. 435) showed that intravenously injected aureomycin reached a level of 2.15 μg per milliliter in the blood in fifteen minutes and had leveled off at 1.88 in one hour. In the saliva, the aureomycin concentration was 0.0146 μg per milliliter in fifteen minutes, and 0.116 at the end of one hour, falling off rapidly in the second hour.

Since fluorides do not offer absolute control of dental caries, it might be assumed that protection will vary from individual to individual. Clapper, Downs, and Heatherman found suggestive evidence that the type of lactobacillus present in a child's saliva may influence caries activity when fluoridated water is consumed, although lactobacillus types are not so important in fluoride-free areas (p. 440). The bacterial flora changes from time to time and fluorides in water (1 ppm) do not appear to influence the type of lactobacillus which will become established. It is quite possible that the type of lactobacillus may be important in all individuals with high caries resistance and of little importance in the individual with relatively little resistance to caries.

In spite of care by the author, editor, and publisher, errors occur in the printed page. Our February issue contained two unfortunate mistakes, one occurring in the office of origin, the other in the process of being prepared for publication. These are listed below.

H. B. G. R.

Errata

In the paper by A. L. Russell, "The Inhibition of Approximal Caries in Adults With Lifelong Fluoride Exposure" (J. D. Res. **32**: 138, 1953), the reference key to the charts in Fig. 1 (p. 141) has been reversed. The striped bars shown for Boulder should be for Colorado Springs, and the checked bars shown for Colorado Springs should be for Boulder.

In the paper by O. Backer Dirks, K. C. Winkler, and J. Van Aken, "A Reproducible Method for Caries Evaluation. III. Test in a Therapeutic Experiment With an Ammoniated Dentifrice" (p. 18), the graphs were misplaced in relation to the legends. The graph with the legend for Fig. 4 should be with the legend for Fig. 1, the graph with the legend for Fig. 1 should be with the legend for Fig. 2, the graph with the legend for Fig. 2 should be with the legend for Fig. 3, and the graph with the legend for Fig. 3 should be with the legend for Fig. 4.

RADIOGRAPHY OF THE TEMPOROMANDIBULAR JOINT

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I. Radiographic Technic

THE method employed in this investigation was a modification of the conventional arrangement known as "lateral oblique transcranial radiography,"[1] of which the disposition shown in Fig. 1 is typical. The subject's head and the cassette are inclined at 15 degrees to the horizontal, while the central ray is arranged to enter the side opposite that being delineated at a point (variously located by different writers) above and behind the ear. The result is, in general, a 15 degree angulation in two planes, and the arrangement has as its objects (a) avoidance of intervening structures which might obscure the joint, and (b) delineation of the condyle in profile; that is to say, the central ray is then presumed to traverse the long axis of the condyle.

The validity of these assumptions will be considered presently, but it is first necessary to point out certain disabilities inherent in the recumbent position as such. First, in all positions of the jaws except that of centric occlusion, it is almost inevitable that the mandible will be deflected slightly to the opposite side by its contact with the cassette. Second, the position is one in which precise orientation of the head is difficult to observe and control. Third, although interest in the rest position of the mandible is comparatively recent, the undoubted value of records of this position in diagnosis and treatment, not only in cases of temporomandibular dysfunction but also in routine orthodontic and prosthetic practice, now calls for a technic in which the mandible is completely unrestrained. Formal definition of physiologic rest position of the mandible and of free-way space very properly includes reference to orientation of the head, for rest position and the vertical dimension of the intermaxillary space, as well as centric relationship of the resting mandible to the cranium and the relation of the condyles to the glenoid fossae, change as the head is tipped forward, backward, or to the side.

In the rest position of the mandible, the subject is presumed to be standing or sitting erect.[2, 3] The head is thus oriented in Frankfort horizontal. The adoption of this position as a standard one in temporomandibular radiography carries the incidental advantage that it is an anatomic, anthropologic, and orthodontic convention of long standing and general utility.

A. ORIENTATION IN RELATION TO CONDYLE AND FOSSA

One of the merits of the Lindblom[3] apparatus is that it is designed for temporomandibular radiography with the head in the erect (norma lateralis) position. This apparatus in the form in which it is supplied was used in the preliminary investigations, and with modifications for the remainder of the

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work. As shown in Fig. 2, it is intended to achieve the conventional 15°/15° orientation of cassette and central ray, and a standard target-film distance of 20 inches in an automatic manner, or at least without the necessity for direct reference to angular scales or other measurements.

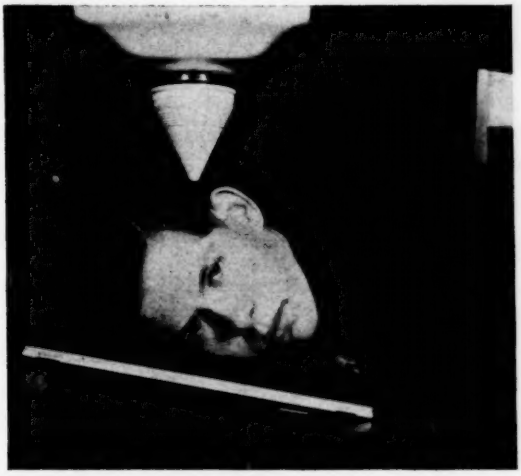


Fig. 1.

Fig. 2.

Notwithstanding the general merit of the apparatus, a certain slackness in fit of the sliding parts and variations in alignment when the assembly is transferred from one side to the other render it incompletely constant in performance. Orientation of the x-ray tube by simple inspection was found to yield equally consistent results, but the headrest and the cassette holder were retained throughout the investigation. Two considerations prompted an early inquiry into the happy fortuity of the assumptions that the 15 degree angulation avoids intervening structures and delineates the condyle in profile. As for

the second of these, it seemed improbable that wide variations in the shape and position of other parts of the skull should not apply also to the temporomandibular joint, and this doubt was strengthened by the results obtained with different patients when the standard angulation was kept constant for all. The quality of delineation of the joint not only varied widely, but was unpredictable.

To confirm the suspicion that the occasionally unsatisfactory radiographs were the result of anatomic variation, rather than of defects in radiographic technic as such, a random selection of twenty-six mandibles was assembled and the orientation of the long axis of the condyles on each was determined, with the results shown in Table I.

TABLE I
INCLINATION IN DEGREES OF LONG AXIS OF CONDYLES TO FRONTAL AND HORIZONTAL PLANES IN 26 MANDIBLES

	FRONTAL		HORIZONTAL	
NO.	LEFT (1)	RIGHT (2)	LEFT (3)	RIGHT (4)
1	9	7	–3	–3
2	12	12	9	9
3	12	12	4	8
4	0	4	–8	–6
5	11	10	–17	–15
6	20	20	0	9
7	12	8	0	0
8	12	13	0	0
9	18	18	12	16
10	25	25	8	5
11	18	17	19	10
12	14	15	0	3
13	5	10	–2	0
14	10	10	10	5
15	10	10	5	0
16	8	0	–2	0
17	20	10	20	15
18	10	15	0	3
19	10	20	–4	–10
20	0	15	–5	0
21	20	30	10	10
22	12	20	–3	0
23	5	4	5	3
24	14	18	0	2
25	15	16	3	4
26	10	25	8	10

Similar explanations for the variable delineation achieved by temporomandibular radiographs have been advanced by Maves,[4] Schier,[5] and Block,[6] but no experiments or measurements were reported. Therefore, it seemed desirable to substantiate these explanations, and if possible to determine the actual magnitude and frequency of the anatomic variations.

The observations reported in Table I are summarized in Table II.

From these measures it can be inferred, in general, that:

1. Orientation of the condyles approaches closely to bilateral symmetry over the whole range of inclinations in both planes.

2. Although the widely accepted mean of 15 degrees for condyle orientation to the frontal plane is confirmed for practical purposes by the observed

TABLE II
STATISTICAL SUMMARY OF TABLE I

	FRONTAL		HORIZONTAL	
	LEFT (1)	RIGHT (2)	LEFT (3)	RIGHT (4)
Mean	12.0	14.0	2.7	3.0
Range	0 to 25	0 to 30	−17 to 20	−15 to 16
Standard deviation	5.9	7.0	8.0	7.0
Standard error of mean	1.2	1.4	1.6	1.4
Mean differences	2.0		0.3	
Correlation	0.59		0.87	

means of 12 degrees and 14 degrees in this series, the conventional mean of 15 degrees for inclination to the horizontal plane is invalid if the observed mean of 3 degrees in the twenty-six mandibles studied is acceptable as a criterion.

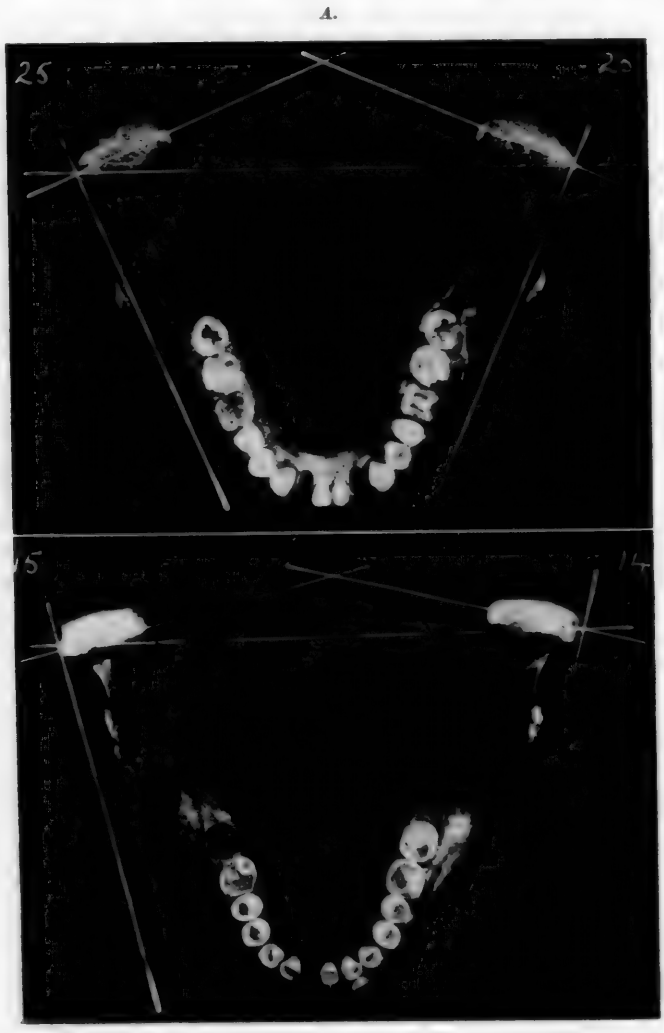
3. Occasional inability to delineate satisfactorily the condyle for different subjects (or even on opposite sides of the same subject) when using a standardized orientation can be explained in terms of such individual anatomic differences as those here described.

Apart from the technic of Schier,[5] there exists no immediate method by which these wide individual variations may be anticipated, and even when poor delineation in a radiograph indicates that the direction of the central ray has not coincided with that of the condyle and fossa, the direction of the error is still not known. The observed high positive correlation and small mean differences between left and right sides are, indeed, sufficient to justify the common assumption of bilateral symmetry in the condyles as in other anatomic features. Therefore, an angulation of the central ray which produces clear delineation on the left will generally be appropriate also for the right, but the initial difficulty remains.

In describing his solution of this problem, Schier stated, in effect, that if the device holding the film cassette is capable of universal adjustment so that the cassette can always be placed in close contact with the side of the face and the angle and lower border of the mandible, it will then take up a plane of orientation at right angles to the long axis of the condyle on that side. This result is not only desirable in itself, but it enables the central ray to be appropriately directed. On this theory, subjects with tapering faces will have, in general, a high condylar inclination, while those with broad mandibles and parallel-sided faces will have a low condylar inclination. Block[6] also observed this relation.

In general, it was found that the long axis of the condyle is, in fact, so inclined to the frontal plane as to form approximately a right angle with the general direction of the lower border of the horizontal ramus. Fig. 3, *A* illustrates a concordant case, while Fig. 3, *B* illustrates a discrepant one. That is to say, if the central transcranial ray were directed perpendicularly to a cassette placed in contact with the region of the right condyle and the angle and lower border of the mandible of the subject (Fig. 3, *A*), it could be reasonably expected to record a clear profile image of the condyle. Application of the same procedure to the other subject (Fig. 3, *B*) would inevitably result in poor delineation.

A similar relation for the horizontal inclination of the condyle is also apparent. A line perpendicular to the long axis of the condyle and projected downward from its lateral margin tends to touch the lower border of the mandible in the region of the angle. Such a construction has been applied to



A.

B.

Fig. 3.

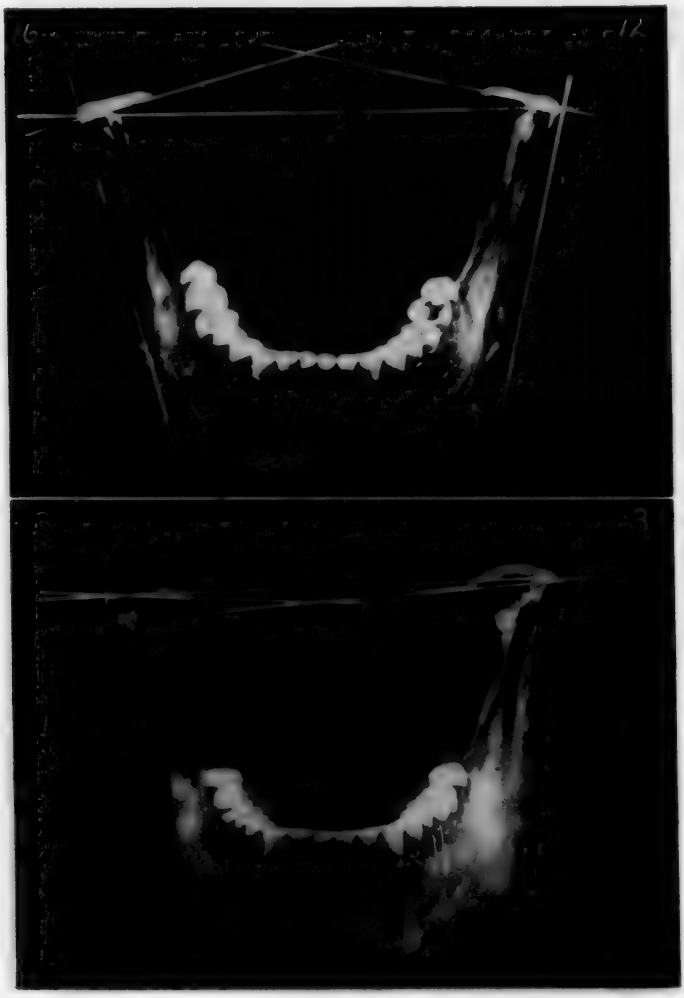
Fig. 4, *A* to illustrate the concept. On one side the coincidence holds, on the other it does not. An even greater and bilateral discrepancy is shown in Fig. 4, *B*.

Over the whole series, however, a rough correspondence in both frontal and lateral aspects does exist, but practical application of this finding to radio-

graphic technic breaks down precisely in those highly discrepant and abnormal cases in which a guide to angulation is most urgently needed.

It should be mentioned in passing that the precise orientation of the cassette is not in itself very significant; it is only when a perpendicular to its surface is postulated on anatomic grounds as a suitable direction for the central ray that the question is important. That only slight distortion is produced when the ideal alignment of the cassette is departed from will be clear by reference to Fig. 5. An error of the same magnitude in directing the central ray is much more serious.

A.



B.

Fig. 4.

A less direct, but more accurate, method of predetermining condylar angulation is that described by Maves.[4] It necessitates two preliminary radiographs of the cranium, one from the posteroanterior (dorsoventral) approach, and one from the submentovertical (cranio-axial) position. These are not intended to delineate the joint as such, but together they provide an outline of both condyles from which their inclination to frontal and horizontal planes can be directly measured.

Arrangements of tube, subject, and cassette are shown in Figs. 6 and 7. The resulting radiographs and the construction for determining condylar angulation are shown in Figs. 8 and 9.

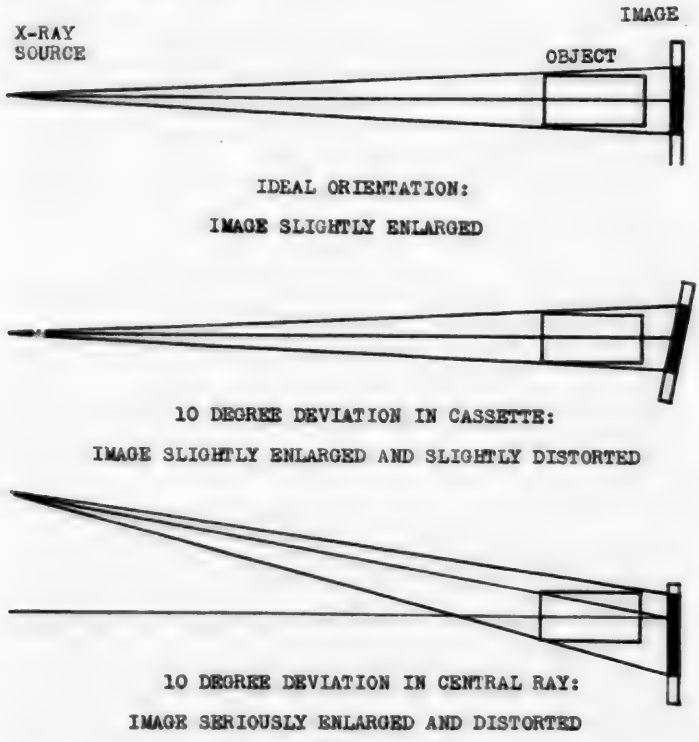


Fig. 5.

This otherwise excellent method of determining condylar angulation involves an additional step. It is impracticable with a dental x-ray unit, and, if a series of six or more transcranial oblique radiographs are to be obtained subsequently, it may expose the patient to excessive radiation. It is, therefore, of some practical importance to inquire into the frequency with which the method must be resorted to. In my series of over 100 patients in whom a standard orientation was employed without a predetermination of condylar angulation, good delineation was achieved in about 70 per cent, only fair in 20 per cent, and very poor in 10 per cent. It is interesting to observe that this 70 per cent satisfactory delineation corresponds closely to the proportion of cases embraced

Fig. 6.

Fig. 7.

Fig. 8.

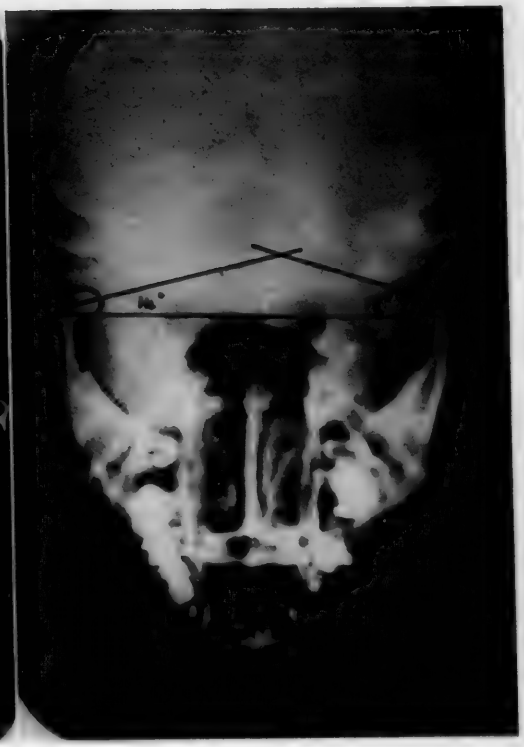


Fig. 9.

by one standard deviation extending on both sides of the mean in the group of condylar angulations reported in Table I, and it is, therefore, a reasonable inference that delineation adequate for diagnostic purposes will result when the angulation of the central ray is within plus or minus 7 degrees* of the actual condylar inclination. For the 30 per cent in which the deviation is greater, a preliminary determination of the optimum angle of incidence would appear to be desirable. In the following discussion, however, it will become apparent that this conclusion requires an important qualification.

It should be added that the technic is not free from difficulties. In both positions the condyles are a considerable distance from the film, and definition suffers accordingly. Even with a 20 degree departure from a strictly horizontal posteroanterior view, it is impossible to avoid obstruction by the mastoid process.

B. ORIENTATION IN RELATION TO INTERVENING STRUCTURES

Before proceeding to a discussion of anatomic obstructions, a further general point on orientation requires clarification. It has been mentioned previously that the direction of the central ray is by some writers defined in degrees of inclination to certain planes, and by others in terms of linear measurements above and behind the external auditory meatus on the side of the skull nearest the tube. Table III shows typical recommendations.

TABLE III

AUTHORITY	ABOVE EXTERNAL AUDITORY MEATUS (CM.)	BEHIND EXTERNAL AUDITORY MEATUS (CM.)
Lindblom[3]	6.0	2.0
Block[6]	6.5	1.3
Gillis[7]	4.0	–4.0
Riesner[8]	5.1	–1.3
Brandrup-Wognsen[9]	3.6	2.4

In view of the doubtful reliability of angular scales on many portable dental x-ray units, the method of linear measurement would appear to have practical advantages. But apart from the evident lack of agreement on where the point of entry should be located, a moment's reflection will reveal that there can be only the roughest correspondence between the two methods of describing orientation, because the point of entry for any given angulation of the central ray will vary with the width of the patient's head. Although this is self-evident, it was nevertheless considered of interest to determine an approximate norm and range for cranial width, and to demonstrate the effect of this variable on the point of entry for a given angulation of the central ray.

Measurements of cranial width across the condyles were made on twenty-five young adult males by means of an ordinary dental face-bow, with the following results (in inches):

5¼	5½	5	5¼	5¾	5¼	5½	5½	
5½	5½	5¼	5¾	5¼	5¼	5½	5	
5¼	5	5	5¼	5½	5	5	5¼	5½

Mean: 5⅛ Range: 5 to 5¾

*Standard deviation derived from Table II.

Fig. 10 has been drawn on the basis of the range in these observations. It may be regarded as a conservative statement of the discrepancies which may occur between linear and angular measurements because, if a larger sample had been taken or if women and younger persons had been included, the bottom end of the range would have been considerably lower. Moreover, the diagram has been drawn with the mid-sagittal planes of the two skulls coincident. If both skulls had been represented with the right sides coincident (as indeed they would be in practice, so that the face would make contact with the cassette when using a fixed target-film distance) the difference between dimensions *A* and *B* would have been greater. But, on the basis of the observed values, and assuming the desired angle to be 15 degrees, it is evident that the point of entry for the narrow skull (*A*) would be 3.5 cm. and for the wide skull (*B*), 4.4 cm. above the external auditory meatus. Exactly similar considerations apply to the inclination of the central ray to the frontal plane, and hence to the location of the point of entry behind the meatus.

It can be concluded that specifications for the direction of the central ray can have exact meaning only when expressed in degrees of inclination to frontal and horizontal planes.

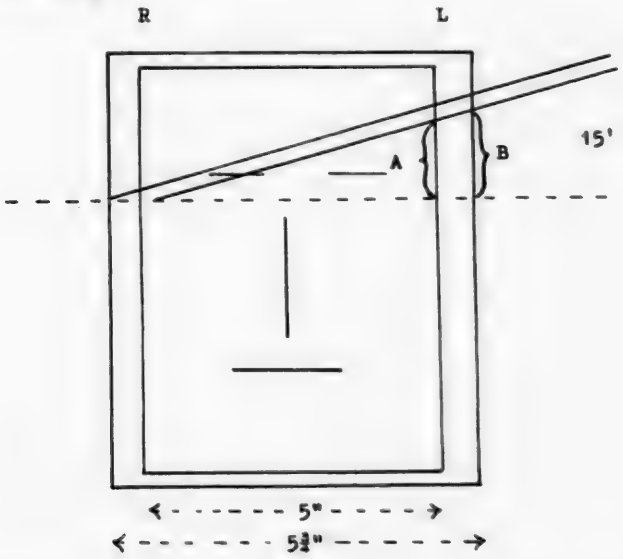


Fig. 10.

It is commonly assumed that a central ray angulation of about 15 degrees to the horizontal plane not only traverses the long axis of the condyle, but is dictated also by the necessity for avoiding obstruction by the body of the sphenoid bone and the petrous portion of the temporal bone on the side adjacent to that being pictured. Indeed, Lindblom's construction for the path of the central ray is almost entirely determined by its relation to these structures.

By sectioning a skull which was judged to be of normal proportions, it was possible to confirm Lindblom's findings. A central ray which is inclined

15 degrees to both planes just clears the petrous portions of the temporal bones on both sides.

For condyles having very low inclinations, clear radiographic delineation by the oblique transcranial approach would, therefore, appear to be physically impossible.

It follows that nothing is gained by separate predetermination of condylar inclination by the use of posteroanterior and cranio-axial films when these reveal inclinations of less than about 10 degrees to either or both planes.

Technical Data.—All radiographs were made on fast film in 9 × 12 cm. cassettes fitted with high speed intensifying screens and a Lysholm stationary diaphragm. The exposed portion of the film was reduced to a circular area of 2½ inches in diameter by means of a lead diaphragm having an aperture of ⅝ inch placed inside the cone of the x-ray tube. The resulting reduction of scatter and secondary radiation greatly improves the definition and quality of the image. With a target-film distance between 13 and 20 inches, exposures with a standard dental x-ray unit working at 10 M.A. and 65 kv. ranged from 4 to 6 seconds. For patients who had not recently been subjected to radiation for other purposes, a total exposure of 400 M.A. seconds was regarded as the maximum safe dose. This permitted a total of eight separate exposures (four on each side), though with very few exceptions the exposures were limited to three on each side, this number being found adequate for general diagnostic purposes.

Routine investigation comprised three exposures of each joint with the mandible in the following positions: (1) rest; (2) occlusion; and (3) either incision or wide open without strain.

The patient's head was supported erect on a firm headrest, and in the case of nervous subjects or children the head was secured with a restraining bandage. The head was oriented in median sagittal and Frankfort planes, and the lower edge of the cassette was always horizontal. Such a standard orientation of head and cassette makes it possible correctly to relate the joint to the cranium as a whole, and to determine the inclination of the articular surface of the fossa and the precise direction of condylar movements.

Although this investigation was intentionally carried out with apparatus normally available in a dental office, there is no doubt that the standard dental x-ray machine is quite inadequate for temporomandibular radiography. A tube of at least four or five times the output would permit exposures of about one second in combination with a Potter-Bucky diaphragm, and would produce radiographs of greater clarity and diagnostic value.

Validity.—If apparent abnormalities of joint structure resulted from mere accidents of radiographic technic, diagnosis based on radiographs would obviously be impossible. The uncertainties and limitations of the radiographic method should therefore be defined, and an attempt, however difficult, should be made to formulate criteria of validity.

Many writers will be found to state that temporomandibular radiographs are of little value in diagnosis, but the limitations are rarely described in detail. In its application to this region, the virtue of the radiographic method lies in its ability to record the relative position, and, hence, changes in relative position, of adjacent radiopaque structures which lie in the same plane. Of soft tissues,

on the condition of which information may be urgently required, it makes no record. Moreover, the condyle and glenoid fossa, like other bony structures, are three dimensional; their shadows are liable to enlargement, distortion, poor definition, and spurious changes in spatial relation with changes in orientation. Reduced to its simplest terms, the problem is that of securing an image of the condyle and glenoid fossa in true profile. The difficulties of achieving this have been sufficiently discussed, but it is appropriate to mention here two examples of departures from valid representation: one in which, so to speak, the radiograph seems to say too much, the other in which it says too little.

It is impossible radiographically to render the space between the condyle and the bony fossa wider than it really is, but an inappropriate direction of the central ray will render it narrower. This possibility is of interest when one is reporting on the dimensions of the joint space generally, but it is of particular significance in establishing or eliminating a diagnosis of retroposition of the condyle. If the central ray enters the opposite side of the head at a point which is too far back, the tympanic plate will be thrown forward and the space posterior to the condyle will appear indistinct, narrow, or even obliterated. It is fortunate that the more extreme errors of this kind can be recognized by the fact that the external auditory meatus is also thrown forward and appears in improbable proximity to the fossa, or may even appear to encroach on it.

Deviation of the central ray from the appropriate direction can result also in enlargement of the condyle, a double image of the fossa (due to lack of superimposition of its lateral and medial borders), and imperfect definition of the boundaries of both. In these circumstances, the condyle will appear to lack a clearly defined cortical layer, and may even appear "eroded" when, in fact, it is normal. Knowledge of the very great difficulty of demonstrating true erosions radiographically serves as a check on this particular form of radiographic invalidity.

Such artifacts, together with the well-known inherent limitations of radiography applied to any part of the body, must be considered in deciding the validity of temporomandibular radiographs. Here, as in other regions, the primary criterion of validity is clarity. If a radiograph of the joint is clearly defined, it can be taken as a valid representation of the spatial relations of condyle and fossa in that plane. If, as a result of movement, inappropriate angulation, shadows of intervening structures, or other faults, it is not clear it cannot serve as the basis for any statement on condyle-fossa relations, much less for a diagnosis either of normality or of arthrosis.

Reliability.—In the sense of insuring that identical radiographs of the same joint can be produced at will on successive occasions, reliability is not difficult to achieve. This is, indeed, fortunate. It has been suggested already that the chief virtue of temporomandibular radiography lies in its ability to record relative positions, rather than pathologic processes. If radiographs are to be of practical value in revealing anatomic changes produced during, or as a result of, treatment, it is essential that positional errors should remain constant for each patient.

The highly accurate and reproducible orientation characteristic of machines, such as the Bolton-Broadbent or Margolis cephalometers which picture the whole

cranium, can be shown in practice to be unnecessary in radiographing the restricted area of the temporomandibular joint. Repeated tests during the course of this investigation indicated that observance of a few simple precautions yields adequate reliability. It is sufficient to orient the head in the median sagittal and Frankfort planes by inspection, to place the cassette in close contact with the side of the face, to maintain a fixed target-film distance, to direct the central ray to the opposite condyle by inspection, and to record the angulation of the tube or the point of entry on each occasion. The mechanical devices employed to facilitate these arrangements matter little in themselves.

II. Radiographic Characteristics of the Normal Joint

A. DIMENSIONS OF THE INTERARTICULAR SPACE

Difficulty and conflict in radiographic interpretation have no doubt been aggravated by the absence of published norms derived from an adequate number of observations. A knowledge of normal shapes of condyles and fossae can be obtained, of course, from a direct study of dry specimens. Since their direct appearance and their representation in good radiographs are similar, it is unnecessary to describe them here. Moreover, these shapes have been widely reported in the literature, the varying inclinations of the articular surface of the fossa have been measured, and fossa outlines and depths typical of Angle's three classes of occlusion have been established.

Literature on the relations of condyles to fossae is less extensive. It is generally appreciated that examination of skulls to determine such relations can be misleading because interarticular soft tissues are absent, bones shrink on drying, and occlusion of teeth, even when these are present in sufficient numbers, is uncertain. Actual dissection of a structure as loosely articulated as the temporomandibular joint is not free from the hazards of post-mortem changes in relations, although the dissections of Shapiro and Truex[10] closely approach the ideal.

For these reasons it was considered most profitable to direct attention to the joint space, rather than to its bony elements. The characteristics which follow, although supported by the investigation as a whole, were derived from a separate study of thirty healthy, young, adult males of an average age of 24 years. All had a reasonably full complement of natural teeth free from marked occlusal anomalies, and all were free from joint symptoms.*

Four exposures of each joint (left and right) were made in the following positions: rest, occlusion, incision, and open. These yielded a total of 240 radiographs in this series.

Tracings of left and right joints of some of these normal subjects in a position of occlusion are reproduced as Fig. 11. For ease of comparison all joints, whether left or right, have been similarly oriented. The characteristics of normality to be described are derived from the complete series of tracings.

Without deduction for slight magnification, the space occupied by interarticular soft tissues averages 3 mm. in width when measured directly above the

*Since clicking of the joint on movement is regarded by many writers as pathologic, it is interesting to notice incidentally that clicking could be elicited in thirteen of these subjects.

condyle, as shown at *B* in Fig. 12. The smallest dimension of the interarticular space averages 2 mm. and is found directly opposite the articular slope at *A*. Posteriorly at *C* the space is more uniform and is intermediate in width, averaging 2.5 mm. Standardized directions for recording such measurements are suggested in Fig. 12. Line *B* at 90 degrees to Frankfort is drawn so as to pass

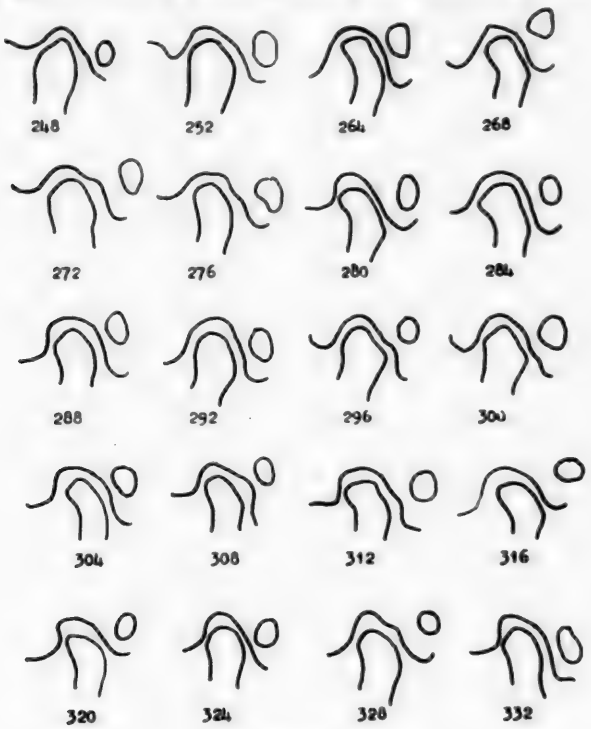


Fig. 11.

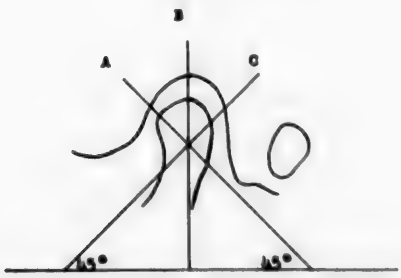


Fig. 12.

through the most superior point on the condyle; line *A* is directed forward at 45 degrees to Frankfort in such a way as to traverse the narrowest part of the articular space; line *C* is also drawn at 45 degrees to pass through the intersection of the other two. The averages just quoted for the three dimensions of the joint space are derived from Table IV.

TABLE IV
APPROXIMATE DIMENSIONS OF THE INTERARTICULAR SPACE MEASURED IN THE DIRECTIONS SHOWN IN FIG. 12 (SERIES OF 30 NORMAL ADULTS, POSITION OF OCCLUSION AS SHOWN IN FIG. 11)

NO.	(A) MM.	(B) MM.	(C) MM.
1	2.0	2.0	2.0
2	2.0	3.0	2.0
3	2.0	4.0	2.5
4	2.0	3.0	2.0
5	2.0	1.5	2.0
6	2.5	2.0	2.0
7	2.0	3.0	3.0
8	2.5	2.5	2.5
9	1.5	2.5	2.5
10	2.5	3.0	2.0
11	2.0	2.0	3.0
12	1.5	2.5	3.5
13	2.5	2.0	2.0
14	2.0	3.5	1.5
15	2.0	2.5	2.5
16	2.0	3.5	2.5
17	1.5	3.0	1.5
18	2.0	4.0	3.0
19	2.0	3.0	1.5
20	3.0	3.0	2.0
21	2.0	2.0	2.0
22	1.5	3.0	2.0
23	2.5	2.5	2.0
24	2.0	2.5	1.5
25	3.0	3.0	3.0
26	2.5	2.5	2.5
27	1.0	3.5	1.5
28	2.0	3.0	2.0
29	1.5	2.5	2.5
30	2.5	3.5	2.5
31	2.0	2.5	2.0
32	2.0	3.0	2.5
33	3.0	2.5	2.5
34	2.0	2.5	3.0
35	1.5	3.0	2.5
36	3.5	3.0	2.5
37	1.5	3.5	2.0
38	1.0	3.0	1.5
39	2.0	4.0	3.0
40	1.0	3.0	2.5
41	2.5	4.5	2.0
42	2.0	3.0	3.5
43	1.5	3.0	2.0
44	3.0	3.5	2.0
45	2.0	4.0	3.0
46	1.0	2.0	2.0
47	2.0	3.0	3.0
48	2.5	4.0	3.5
49	2.0	3.0	3.5
50	2.0	3.0	3.5
51	3.0	4.0	3.0
52	1.5	2.5	3.0
53	3.0	2.5	1.5
54	4.0	3.0	2.0
55	1.5	2.5	1.0
56	3.0	3.5	2.5
57	2.0	3.0	2.0
58	3.5	2.5	2.0
59	3.0	4.0	2.5
60	2.0	3.0	2.0
Means	2.0	3.0	2.5

The term "approximate symmetry" is suggested as appropriate for describing normal condyle-fossa relation in occlusion as revealed in this investigation. A brief explanation is called for and it is also necessary at the outset to deal unequivocably with a common misconception. Maves[4] stated that, "In order to differentiate between the normal and the abnormal positions of the head of the condyle in the fossa, the normal as given by Goodfriend and others was adopted; i.e., when the upper anterior surface of the head of the condyle rests near the *lower* posterior border of the articular eminence with the meniscus between serving as a cushion, we have the normal position of the joint." This interpretation has been accepted by many dentists (though not by anatomists), and it has been especially welcomed by those who have sought to treat temporomandibular arthroses by moving condyles forward. Since this statement is in serious conflict with one of the main conclusions of the present study, and since treatment is not infrequently based on such a concept of normality, it would be quite inexcusable to avoid critical reference to it here.

If a large space posterior to the condyle and an intimate relation between the condyle and the lower portion of the articular slope of the fossa be accepted as characteristics of a normal, terminal, functional position, that is, coinciding with centric occlusion, then a large proportion of perfectly normal joints can be described as having posteriorly displaced condyles. That truly retroposed condyles should be brought forward to a normal anatomic relation is not disputed; neither is it denied that even when condyle-fossa relations are normal, relief of pain has sometimes, although often only temporarily, followed the establishment of an occlusion which enforces a more forward position. If symptoms can be relieved in this way without incurring any functional disability, the procedure must be commended, but it should be recognized as a purely therapeutic measure, the rationale of which is not understood at present.

It will now be evident that the nature of this articulation calls for careful description. It can be conceded that the most intimate area of the articulation occurs between the anterosuperior articular surface of the condyle and the articular slope of the fossa, and that between these two areas the thinnest part of the meniscus is interposed. The general upward and forward direction of muscular forces on the joint, the histologic structure of the meniscus in its different parts, and the thinness of the bone between glenoid fossa and middle cranial fossa, all point to the articular slope as being the main pressure-bearing region of the joint. But, this is merely to say that the essential part of the articulation is not between the most superior surface of the condyle and the roof of the glenoid fossa; it does not imply that the condyle is almost at the bottom of the articular slope, or that it is only remotely related to the general concavity of the fossa.

A confident statement on the exact relation of the parts could be made only after careful dissection of an adequate number of specially prepared cadavers having a reasonably full complement of teeth in normal occlusion. It would probably be necessary to wire the jaws together in occlusion before preparing the specimens and to maintain them thus during the dissection. In view

of the difficulties in assembling and preparing the material in sufficient quantity, it is doubtful whether this painstaking task has ever been undertaken. We do know, however, that the radiographic appearance is normally one of symmetry, and that radiographic appearance cannot differ except to a small extent from anatomic facts. On the basis of radiographic evidence one, therefore, could not regard a diagnosis of retroposition of the condyle as justified unless its relation to the fossa was posterior of symmetry. Although Gillis,[7] Thompson,[11] and Harvey[12] make no reference to this feature, their radiographs or tracings and those of many other investigators show this approximate symmetrical relationship quite as well as my own.

Owing to the existence of normal variations, it is impossible to define exactly the place on the articular slope with which the condyle is normally in most intimate relation, for, if the condyle is relatively large and the fossa shallow, this region will of necessity be located downward and toward the articular eminence; if the condyle is small, the fossa deep, and the articular surface steeply inclined, the region of most intimate relation will be more superiorly placed in the fossa. It would seem, therefore, that precise description of normal or abnormal condyle-fossa relations would be best attempted in terms of the relative dimensions of the joint space in the three directions already suggested in Fig. 12. If such a convention were adopted, these relations could be expressed by a simple numerical ratio. For example, the mean ratio of the present normal observations would be A2: B3: C2.5. A ratio such as A2: B6: C2.5 would be typical of a downward displacement, while A4: B2: C1 would signify a retroposition.

B. CONDYLAR MOVEMENTS

1. *From Rest Position to Occlusion.*—For many years before the concept of rest position had become explicit, it had been known that when mandibular opening was limited to a few millimeters at the incisors, the movement was a simple, hingelike one, and that downward and forward translatory movement of the condyle occurred only on wider opening. More recently, this traditional description had been confirmed radiographically by Boman,[13] Thompson,[14] Rosenfeld,[15] and many others. In normal occlusion, and especially in the absence of deep overbite or overclosure, movement of the condyle in passing from rest position to occlusion occurs about the horizontal intercondylar axis, with little or no translatory component.

The results of the present study are in accordance with these findings. Simple axial closure from rest position can be regarded as characteristic of the normal joint. It must be added, however, that the arc described by the incisors is so small as to make the movement radiographically imperceptible at the condyles. Axial closure can be deduced only from the absence of translatory movement. If condyle-fossa relations appear identical or almost identical in rest position and occlusion, the joint is in this respect normal.

The group of thirty normal adults previously referred to provided sixty pairs of radiographs of occlusal and rest positions of the condyle. Of these pairs eighteen were identical, twenty were within 1 mm. of coincidence, and twenty-two lacked coincidence by amounts varying from 1.5 to 2.5 mm. Thus,

two-thirds of the cases fell within 1 mm. of coincidence, but since all members of the group were clinically normal, it would seem prudent to extend the range of normality from identity to 1.5 mm. Approximately three-quarters of the subjects would then be included. In the absence of other criteria, the latter range is offered as a tentative norm for adults.

2. *Translatory Movements.*—Protrusion of the mandible to a position of incision involves a small downward and forward movement of the condyle, the movement obviously varying in magnitude with the anterior overbite and overjet. In the normal joint, wide opening movement without undue effort brings the condyle directly below the articular eminence. Limitation of movement or excessive movement can be recorded radiographically, and such records will add precision and certainty to what may already be evident clinically.

The radiographic appearance of these separate positions (occlusion, rest, incision, and open) is well known. It is only when they are studied together that they yield, as it were, an interrupted kinematic picture of the whole range of functional movement which promises to be of diagnostic value. It was found that in its four positions the condyle maintains a constant separation of about 2 mm. from the articular surface of the glenoid fossa. That is to say, condylar movements within the functional range are normally congruent with the fossa outline. Congruency of movement in this sense was present in fifty of the sixty normal joints investigated.

3. *Characteristics of the Joint in Edentulous Subjects.*—Radiographic examination of twenty completely edentulous adults within the age range of 35 to 65 years failed to reveal any marked departure from normal condyle-fossa outlines and relations. All patients had worn full upper and lower dentures for periods ranging from ten to thirty years, and all had been supplied with new dentures restoring proper jaw separation a few weeks previously. Under these circumstances, evidence of overclosure could not be expected, though it could sometimes be shown with old dentures. The majority of patients had a condyle-fossa relation 1 mm. posterior of symmetry with the new dentures in occlusion.

All patients were free from symptoms and claimed to masticate satisfactorily. Clicking of the joint on opening or closing movements was evident in half of the group. The condyles could be easily palpated in the auditory meatus in a similar number. There was no departure from normal radiographic appearance in the presence of either of these features, and they are, therefore, not regarded as significant.

I conclude that norms for joints of completely edentulous subjects do not differ essentially from those for normal subjects, and that the wearing of full dentures does not in itself produce degenerative changes capable of radiographic demonstration.

4. *Radiographic Characteristics of the Abnormal Joint.*—Since abnormalities can be deduced readily from the previous discussion of norms, they call for only brief mention here. The commoner departures from normality which can be demonstrated radiographically are listed here. They may be unilateral or bilateral.

(a) *Excessive mobility* leading to forward dislocation of the condyle beyond the articular eminence on wide opening.

(b) *Limited mobility* most frequently takes the form of reduced ability to open the mouth, although lateral or protrusive movements may be similarly restricted. It commonly follows severe trauma, such as a blow on the chin, but it is also typical of the recurrent acute phases of long-standing arthritis. The limited opening movement is often atypical and is achieved mainly or entirely by rotary movement of the condyle with little or no translatory component.

(c) *Abnormally large or small articular space.* Following Prentiss[16] and Summa,[17] many writers have claimed that in arthrosis the disc becomes thinned or even perforated, and the condyle more intruded in the fossa. It has been found in this investigation that an inferior displacement of the condyle, and consequently a large articular space, is equally characteristic of dysfunction. In a search of the literature, Connors[18] was the only writer who could be found to mention this possibility and to establish the fact by anatomic dissection.*

(d) *Lack of symmetry* of the condyle in relation to the fossa, and especially retroposition of the condyle in occlusion, has already been discussed. Two varieties of retroposition can be distinguished clinically: one due to simple overclosure such as might follow the prolonged wearing of full dentures or attrition of natural teeth, the other due to direct backward deflection of the mandible by malposed cusps. The latter variety is more commonly productive of symptoms than the former.

(e) *Large translatory movement* of the condyle in passing from rest to occlusal position is of particular interest to orthodontists because it is found most frequently and in its most exaggerated form in children having Class II malocclusion. It is on the presence or absence of this movement that Thompson bases his diagnostic distinction between spurious and true Class II jaw relations. In children, such a movement is rarely, if ever, associated with symptoms; in adults, it is significant in association with retroposition, of which it may give valuable confirmation.

(f) *Lack of congruency of movement* establishes prima facie evidence of atypical function. Presumably it results from slackness of the capsular ligament or failure of the disc to follow translatory movements of the condyle.

(g) *Flat or steep articular surface of fossa.* This study confirms the well-known, though rough, correspondence between the type of occlusion and the steepness of the articular slope of the fossa, Class II malocclusions generally being associated with a steep slope, as reported by Miller.[19] The average inclination of the articular slope to the Frankfort plane is about 50 degrees, the steepest inclination observed being 90 degrees. Inclinations to the occlusal plane, which is conventionally regarded as horizontal by prosthetists, average about 9 degrees less.

Throughout this investigation I have gained the impression, although I cannot establish it statistically, that patients with Class II malocclusions, deep anterior overbite, and steeply inclined fossae are predisposed to temporomandibular dysfunction.

*This article antedates by many years the general application of radiography to the temporomandibular joint. It is remarkable as an early, unprejudiced, and careful, but rarely quoted, study of this region.

COMMENT

The urgent challenge and interest of this field of study arises from the inherent difficulty of evaluating the normal, and departures from it. Individual response to abnormalities of articular anatomy and function is baffling in its variety and uncertainty. In one subject, gross malocclusions and departures from normal articular relations will give rise to no symptoms; in another, no trace of abnormality can be found to account for the most distressing clinical signs and symptoms.

It is clearly beyond the power of radiographic investigation alone to explain these anomalies. That it can, nevertheless, contribute materially to an understanding of temporomandibular joint dysfunction will be evident from the foregoing discussion.

SUMMARY

1. The technical and anatomic difficulties of transcranial oblique radiography are elucidated and a reliable procedure is described.

2. The dimensions, functional relations, and general characteristics of the normal temporomandibular joint as seen in radiographs are established.

3. Typical departures from normal anatomy and function are described and related to clinical signs and symptoms.

REFERENCES

1. Norgaard, F.: Temporomandibular Arthrography, Copenhagen, 1947, Ejnar Munksgaard.
2. Thompson, J. R.: Rest Position of the Mandible and Its Significance to Dental Science, *J. A. D. A.* **33**: 151, 1946.
3. Lindblom, G.: Technique for Roentgen-Photographic Registration of the Different Condyle Positions in the Temporomandibular Joint, *D. Cosmos* **78**: 1227, 1936.
4. Maves, T. W.: Radiology of the Temporomandibular Articulation With Correct Registration of Vertical Dimension for Reconstruction, *J. A. D. A.* **25**: 585, 1938.
5. Schier, M. B. A.: New Technique for Radiography of the Temporomandibular Articulation, *Dental Items Interest* **65**: 324, 419, 1943.
6. Block, L. S.: Diagnosis and Treatment of Disturbances of the Temporomandibular Joint Especially in Relation to Vertical Dimension, *J. A. D. A.* **34**: 253, 1947.
7. Gillis, R. R.: Roentgen Ray Study of Temporomandibular Articulation, *J. A. D. A.* **22**: 1321, 1935.
8. Riesner, S. E.: Temporomandibular Articulation: Its Consideration in Orthodontic Diagnosis, *Internat. J. Orthodontia* **22**: 1, 1936.
9. Brandrup-Wognesen, F.: Metod for kakledsfotografering, *Odontologisk Tidsskr.* **53**: 191, 1945.
10. Shapiro, H. H., and Truex, R. C.: Temporomandibular Joint and the Auditory Function, *J. A. D. A.* **30**: 1147, 1943.
11. Thompson, J. R.: Oral and Environmental Factors in Malocclusion of the Teeth, *Am. J. Orthodont.* **35**: 33, 1949.
12. Harvey, W.: Investigation and Survey of Malocclusion and Ear Symptoms, *Brit. D. J.* **85**: 249, 1948.
13. Boman, V.: Unpublished Thesis. Northwestern University, 1947. Quoted by J. R. Thompson.[11]
14. Thompson, J. R.: Rest Position of the Mandible and Its Application to Analysis and Correction of Malocclusion, *Angle Orthodont.* **19**: 162, 1949.
15. Rosenfeld, L. D.: Investigation of a Treatment for Periodontal Disturbances, *Northwest. Univ. Bul.* **49**: 13, 1949.
16. Prentiss, H. J.: Preliminary Report Upon the Temporomandibular Articulation in the Human Type, *D. Cosmos.* **60**: 505, 1918.
17. Summa, R.: Importance of the Interarticular Fibrocartilage of the Temporomandibular Articulation, *D. Cosmos* **60**: 512, 1918.
18. Connors, M. C.: Tissue Reactions in the Temporomandibular Articulation Following the Loss of Teeth, *D. Cosmos* **67**: 124, 1925.
19. Miller, S. C.: Oral Diagnosis and Treatment, ed. 2, Philadelphia, 1946, The Blackiston Company, pp. 231-258.

THE EFFECT OF MASTICATORY EXERCISE ON THE MAXIMUM FORCE TOLERANCE OF INDIVIDUAL TEETH

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I. Young Adults

NUMEROUS references have appeared in the dental, medical, and physiological literature dealing with the decrease of masticatory function in human beings.[1-19] There seems to be general agreement that modern culinary arts and dietary habits have made vigorous and forceful mastication unnecessary. Lack of masticatory exertion has been associated with degenerative changes in the oral cavity.[20] Some of the influences listed include lack of osseous development,[21, 22] retrograde changes in the gingival tissues, periodontal structures,[23, 30] the salivary glands,[2] decreased functional efficiency of the masticatory apparatus,[20] increased incidence of malocclusion,[24] and increased incidence of dental caries.[16] It has been suggested by some investigators that masticatory exercises are generally desirable and serve to stimulate and strengthen the dental apparatus.[26, 27]

Several studies have been conducted to evaluate the effect of increased masticatory exercise on maximum biting forces. Brekhus, Simon, and Armstrong[32] reported that chewing of a one-half inch cube of paraffin for one hour per day for a period of fifty days increased the maximum biting pressures of the whole mouth by an average of twenty-four pounds. Friel[33] has reported that incisor biting forces could be increased up to 500 per cent in some instances with the aid of proper muscular exercises. Added function could influence the muscles of mastication, the temporomandibular joint, and the supporting structures of the teeth. The supporting structures of the teeth include the periodontal membrane and the alveolar bone. It was the purpose of this investigation to evaluate the effect of increased masticatory exercise on the force tolerance and maximum biting force of individual teeth in an attempt to evaluate more exactly the mechanism of action associated with the increase of biting pressure.

EXPERIMENTAL PROCEDURE

One hundred two young adults (dental students) were used as subjects in this investigation. Their age ranged from 19 to 29 years, the mean age being 23 years. They were divided into one control group and three experimental groups. Each experimental group was supplied with a different chewing medium, namely, a one-half inch cube of paraffin, 1.5 Gm. of an unflavored gum base, and a 2.5 Gm. portion of a commercial chewing gum. The experimental subjects were requested to chew the test medium supplied for three one-hour periods

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daily, during the course of the testing procedure. The control group was requested to refrain from gum chewing. Each subject was also requested to fill out a time schedule indicating exactly when this chewing took place.

Maximum biting forces were determined prior to the initiation of chewing procedures and at regular two-week intervals for a period of six weeks. The Howell-Manly gnathodynamometer was used to determine maximum biting forces.[34] The subjects were seated in a quiet semiprivate room in such a manner that they could not see the instrument readings. They were requested to bite slowly on the force element and finally exert as much pressure as they possibly could. Maximum force readings of individual teeth in the lower right and upper left quadrant were evaluated. A total of approximately 6,000 individual force determinations was accumulated in the course of this testing procedure. The special data obtained during this study included age, height, sex, weight, dietary habits, food preferences, and the side of the dentition preferred in chewing. A superficial examination for caries, periodontal disturbances, and oral pathology was also accomplished. In many instances x-rays were used as a clinical aid for this examination.

RESULTS

The mean maximum force tolerances of individual teeth are presented in Table I. The Chi-square test indicated that there was no significant differences in the maximum biting forces of maxillary and mandibular teeth. The molar and premolar teeth on the preferred and nonpreferred sides were grouped individually. No significant differences were found between these two groups, although slightly higher forces were observed on all teeth on the preferred side with the exception of the first bicuspid.

TABLE I

	TOOTH NUMBER						
	MOLARS		BICUSPIDS		CANINE	INCISORS	
CATEGORIES	7	6	5	4	3	2	1
All teeth Upper right	91	94	78	73	58	43	43
All teeth Lower left	90	98	76	68	54	41	38
Preferred side Upper and lower	95	95	82	68			
Nonpreferred side Upper and lower	86	86	73	68			

All test groups increased in force over the original determinations. This seems to indicate that there is a learning factor associated with maximum bite force determinations carried out under these experimental conditions. After the second determination the control group remained rather constant, while the experimental groups continued to increase in force for the duration of the testing program.

A statistical evaluation of changes of force exerted on all teeth in all groups indicated that there was no statistical difference between the biting force determinations taken initially and every two weeks from two to six weeks. This

was probably due to the fact that no increase in maximum biting force was observed on the anterior teeth and that nonpreferred side of the dentition showed relatively small increases.

The data were then regrouped to include only the preferred side of the dentition. Readings on those individuals who stated no preference for either side of the dentition were omitted. This was done in order to assign the greatest possible value to function. Obviously most function is applied to the side of the dentition on which chewing is accomplished. Figs. 1 and 2 show a comparison of the mean biting forces obtained on the control and commercial gum group at various time intervals.

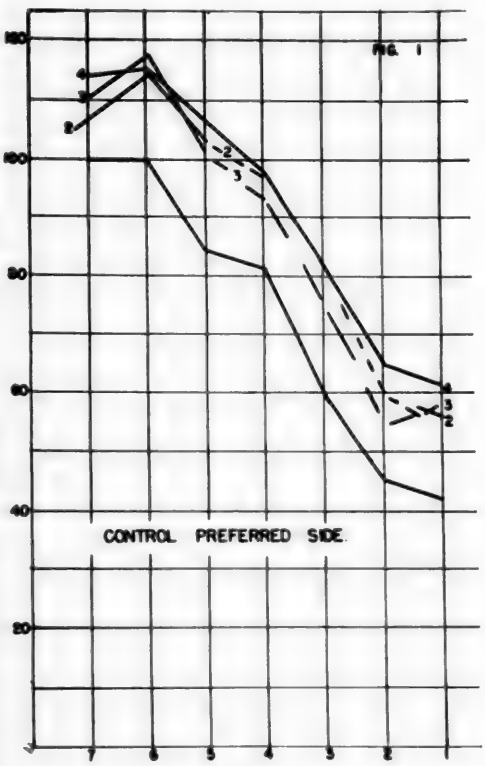

Fig. 1.

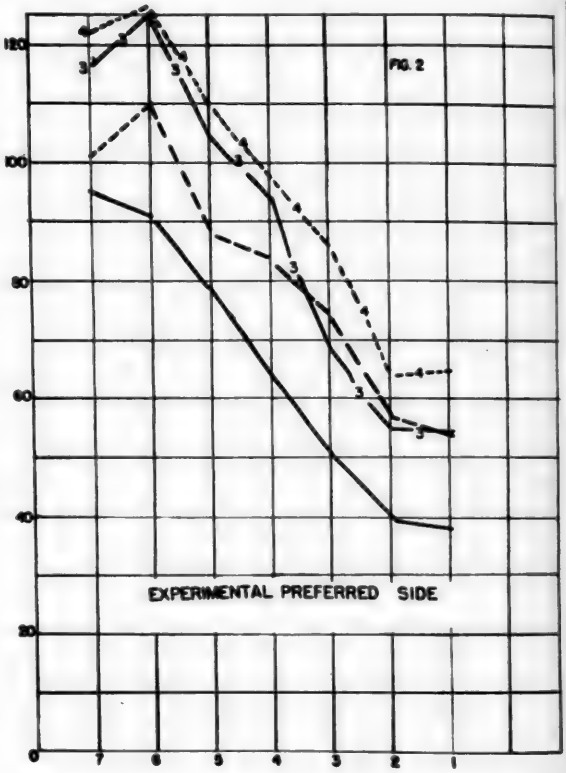

Fig. 2.

It was observed that there was an increase in the maximum biting force in the control group and in all the experimental groups as the test proceeded. There was a progressive increase in all instances except at the six-week period with the hard gum group. This phenomenon cannot be explained satisfactorily, although a distinct lack of requests for further chewing materials were noted for both the hard gum and paraffin groups at this time level. Table II shows a summary of the statistical analyses conducted on the preferred side only. It indicates that there is little change in biting force after a two-week period of added function and that the maximum change takes place at the four-week in-

terval. When values between the initial determinations and the six-week determinations were compared it was found that there was significant increase in the hard gum group and the commercial gum group. The commercial gum group showed the greatest differences. It was concluded from this study that maximum biting forces can be increased on the functioning posterior teeth.

TABLE II

GROUPS	CONTROL		PARAFFIN		GUM BASE		COMMERCIAL GUMS	
	t	SIGNIFICANCE	t	SIGNIFICANCE	t	SIGNIFICANCE	t	SIGNIFICANCE
1st vs. 2nd week	1.807	N.S.	1.360	N.S.	2.010	N.S.	1.330	N.S.
1st vs. 4th week	1.020	N.S.	1.495	N.S.	3.417	1%	2.989	1%
1st vs. 6th week	1.936	N.S.	1.810	N.S.	2.648	5%	3.544	1%
2nd vs. 4th week	1	N.S.	1	N.S.	1.650	N.S.	1.701	N.S.
2nd vs. 6th week	1	N.S.	1	N.S.	1	N.S.	2.092	5%
4th vs. 6th week	1	N.S.	1	N.S.	1	N.S.	1	N.S.

The percentage changes in force over the control levels were also calculated. After two weeks' chewing, the increase varied from 2 to 12 per cent with commercial gum, hard gum, and paraffin, respectively. After four weeks' chewing the percentage increase over the control was 13, 15, and 19 per cent with paraffin, commercial gum, and hard gum, respectively.

DISCUSSION

The added function supplied by chewing various materials such as paraffin, gum base, or commercial gums increased oral force tolerances of the teeth used during mastication. This increased force tolerance may be indicative of an improvement of the teeth and their supporting structures.

SUMMARY

Maximum force determinations have been conducted on 102 young adults for four consecutive two-week periods in an effort to evaluate the influence of added masticatory function in the form of chewing paraffin and gum base and commercial gum on maximum biting forces. It was found that maximum force tolerances were increased by chewing these test materials. No significant increase of maximum biting pressure was observed on the side that was not used for mastication. Maximum effect was noted on the side preferred during mastication. Increases after four weeks' chewing were 13, 15, and 19 per cent over the control level with paraffin, commercial gum, and hard gum, respectively.

II. Children With Orthodontic Difficulties

According to Friel,[33] children with orthodontic problems possess lower average biting forces than those with normal occlusion. It seemed that orthodontic patients would constitute an ideal group in which to study maximum biting force.

A group of fifty children with varying degrees of malocclusion were selected for study. Care was taken to match age, sex, and occlusal disharmonies in both the experimental and control groups. Maximum biting forces were determined using the Howell-Manly type of gnathodynamometer. Maximum force recordings were obtained on the lower right and upper left quadrants on all teeth in occlusion. These were accomplished immediately before chewing procedures were initiated and twelve weeks later. The mean age of both groups was 11.7 years. They ranged from 8 to 14 years of age.

The patients were divided into two groups, an experimental and a control. The control group was requested to make no changes in their oral habits except to refrain from chewing gum. The experimental group was placed on a chewing regime. They were instructed to chew a 2.5 Gm. portion of a commercial gum for three one-hour periods daily, preferably after each meal. Chewing gum was chosen as a test medium because of the general acceptance and cooperation that was noted in previous studies.

Twenty-four of the experimental group and sixteen of the control group were available for re-examination after a twelve-week period.

RESULTS AND DISCUSSION

A summary of the findings appears in Table III. During the course of force determinations, biting on certain teeth resulted in pain. Generally this painful sensation resulted in decreased readings, therefore it was felt justifiable to omit readings where the subject complained of pain for the analysis of the data. In the control group the posterior teeth showed an increase in maximum biting force that amounted to an average of 3.9 per cent. This increase may indicate that a "learning factor" can influence the force readings. In view of previous evidence, this variable plays a smaller part in this group than in a group of young adults. The lesser effect of "learning" may also be due to the complete cooperation that is displayed by most of the children examined, whereas in dealing with young adults a certain amount of anxiety seems apparent that may result in lower biting forces at the initial determination.

TABLE III

	CONTROL				EXPERIMENTAL			
	1ST DETERMINATION		3 MONTHS LATER		1ST DETERMINATION		3 MONTHS LATER	
TEETH	NUMBER	MEAN	MEAN	% CHANGE	NUMBER	MEAN	MEAN	% CHANGE
1st molar	32	64.4	71.4	+10.9	48	59.8	77.5	+29.6
2nd bicuspid	17	54.6	56.3	+ 3.1	22	44.9	60.5	+34.7
1st bicuspid	16	49.5	47.5	− 5.1	25	35.3	44.8	+26.9
Summary posteriors	65	56.2	58.4	+ 3.9	95	46.7	60.9	+30.4
Cuspid	27	36.0	31.2	−13.3	23	26.8	30.0	+11.9
Lateral	28	33.0	26.5	−20.0	25	25.1	25.6	+ 2.0
Central	29	31.2	26.7	−14.4	27	25.6	26.0	+ 1.6
Summary anteriors	84	33.4	28.1	−15.8	75	25.8	27.2	+ 5.4
Over-all summary	149	45.5	43.3	− 9.5	170	36.3	44.1	+21.5

The average force increase in the posterior area for the experimental group amounted to 30.4 per cent. This represents an increase of 26.5 per cent over the control level.

There was a drop in the maximum biting forces of anterior teeth observed in the control group amounting to 15.8 per cent and an increase of 5.4 per cent in the experimental group.

Statistical analysis of the data indicated that there was no statistical difference between initial readings of the control and experimental groups. The increase of maximum biting forces was most significant in the first molar area. An increase significant to the 5 per cent level was demonstrated in the first molar area, whereas the changes in all the posterior teeth merely approached significance. It seemed evident from the data analysis that in children with orthodontic difficulties the first molar area is most effected by added function in the form of gum chewing.

SUMMARY

The effect of added function on the maximum biting forces of individual teeth has been studied in fifty children possessing orthodontic difficulties which were currently under observation or treatment.

Under the conditions of this test, maximum biting forces were increased by an average of 21.5 per cent by added function in the form of gum chewing. The most significant effect was observed in the first molar area.

REFERENCES

1. Leigh, W.: *J. D. Res.* **5**: 197, 1923.
2. Lenhardtson, A.: *D. Cosmos* **58**: 332, 1916.
3. Luther, R. E.: *D. Cosmos* **52**: 551, 1910.
4. Lindsay, R.: *Brit. D. J.* **45**: 1211, 1924.
5. Milberry, G. S.: *D. Cosmos* **72**: 491, 1930.
6. Owre, A.: *D. Cosmos* **68**: 841, 1926.
7. Acland, F. D.: *Brit. D. J.* **46**: 213, 1925.
8. Barker, P. A.: *J. D. Res.* **5**: 99, 1923.
9. Bland, T. M.: *D. Cosmos* **72**: 753, 1930.
10. Bunting, R. W.: *D. Cosmos* **56**: 285 1914.
11. Crane, W. A.: *Brit. D. J.* **46**: 514, 1925.
12. Doherty, J. W.: *Brit. D. J.* **46**: 514, 1925.
13. D'Alise, C.: *Internat J. Orthodontia* **18**: 1038, 1932.
14. Harris, L. A.: *J. D. Res.* **7**: 213, 1927.
15. Sweet, C. A., Watson, R. G., and Stafford, H. E.: *J. A. M. A.* **94**: 544, 1930.
16. Walse, J. J.: *D. Cosmos* **54**: 87, 1912.
17. Ayrappa, M.: *D. Cosmos* **55**: 651, 1913.
18. Bodecker, C. F.: *D. Cosmos* **67**: 543, 1925.
19. Bodecker, C. F.: *D. Cosmos* **68**: 425, 1926.
20. Schutzman, A. M.: Irregular Teeth, Causes, Effects, Treatment and Prevention, Baton Rouge, La., 1934, Franklin Press.
21. Miner, L.: *Internat. J. Orthodontia* **18**: 549, 1932.
22. Obrey, L. J.: *Internat. J. Orthodontia* **22**: 1095, 1936.
23. Leonard, H. J.: *J. D. Res.* **68**: 242. 1926.
24. Sweet, C. A.: *D. Cosmos* **72**: 1159, 1930.
25. Swinehart, E. W.: *D. Cosmos* **69**: 903, 1927.
26. McKeag, R. H.: *Brit. D. J.* **49**: 346, 1928.
27. Stillman, P. R.: *D. Cosmos* **66**: 1042, 1924.
28. Waugh, L. M.: *J. D. Res.* **3**: 31, 1921.
29. Campbell, H.: *Lancet* **2**: 84, 1903.
30. Hartzell, T. B.: *D. Cosmos* **53**: 513, 1911.
31. Hyatt, T. P.: *D. Cosmos* **73**: 283, 1931.
32. Brekhus, P. J., Armstrong, W. D., and Simon, W. J.: *J. D. Res.* **20**: 87, 1941.
33. Friel, E. S.: *Internat. J. Orthodontia* **11**: 377, 1925.
34. Howell, A. H., and Manly, R. S.: *J. D. Res.* **27**: 705, 1948.

A SIMPLIFIED INSTRUMENT FOR MEASUREMENT OF FOOD PLATFORM AREA

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AN INSTRUMENT developed by Yurkstas and Manly for measurement of occlusal contact area (food platform area) was found to be of value in predicting the effectiveness of a natural dentition for mastication of food.[1, 2] Subjects were instructed to bite upon a special strip consisting of plastic fused between two layers of soft opaque wax. After the occlusal surfaces of the molar and premolar teeth had been registered on this wax, the light transmission of the registration was recorded in a special instrument. The instrument consisted of two units: a cylinder containing a light bulb and two photovoltaic cells, and part of a commercial light meter having resistance controls and a galvanometer.

The original equipment had been used at considerable distances from the laboratory; it was too heavy and bulky for convenient shipment, and was rather costly for duplication. It seemed to us that the equipment could be simplified and reduced in cost.

The reduction in over-all size and weight required changes in the optical design and in the source of light employed. The previous model had a long aperture for approximately collimating the light falling upon the wax; if diffusion disks could be used, the minimum length from the light source to the photoelectric cell could be shortened. Cost could be reduced by use of a special electronic circuit, which would eliminate need for precision electrical equipment. Both weight and cost could be lowered by omitting the cylinder.

The final design of the instrument produced an apparatus which weighed one-tenth as much as the original, cost about one-seventh as much, and could be packed into a space one-eighth as large.

DETAILS OF INSTRUMENT CONSTRUCTION

The circuit used is a variation of one shown in the *RCA Phototube Manual*.[3] The modification not only makes use of the high sensitivity in balancing quantities of light, but also permits balancing over a wider range of light intensity. Two vacuum type phototubes are connected in series, and the lead connecting the tubes is joined to the grid of a power pentode. The output from the pentode serves to operate a milliammeter. The calibration of light transmission is achieved through a potentiometer in the circuit which controls a light placed adjacent to one of the photocells within a light-tight cover; thus, the light incident through the wax onto one photocell is balanced by increasing the light which reaches the other photocell from a small light bulb. The wiring diagram is presented in Fig. 1, along with a list of parts.

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The source of light for the registration consists of three 6 to 8 volt bulbs mounted ½ inch from the translucent baffle, which consists of a layer of 3/16 inch white Plexiglas. The wax holder lies between this baffle and a second identical baffle mounted parallel 5/8 inch apart. Behind the second baffle is the phototube No. 929 (RCA). The control phototube is identical. The same carrier assembly is used as in the previous instrument. The light bulb adjacent to the control phototube is connected in series with two 50 ohm potentiometers across the secondary of the filament transformer. One of these potentiometers serves to set the instrument on zero with wax having no tooth registration; the other provides a reading of food platform area in square millimeters after the registration is taken. The plate circuits of the phototube and the amplifier tube are battery-operated in order to eliminate drift. One model of area meter has been built for battery operation requiring a 4.5 volt A battery and a 7.5

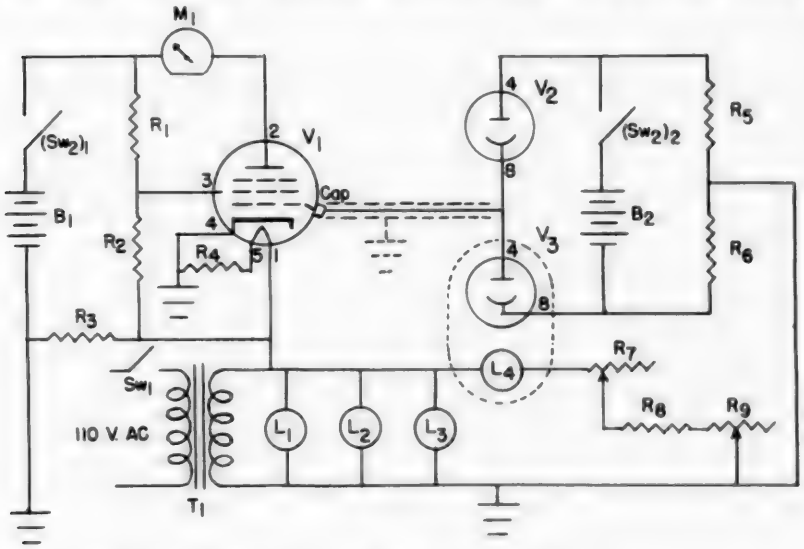

Fig. 1.—Parts list and diagram for modified mastication meter.

Parts List

R_1, 350 ohm
R_2, 220 ohm
R_3, 490 ohm
R_4, 1.5 1 watt
R_5, 3.2 meg.
R_6, 3.2 meg.
$R_{7,9}$, 50 variable
R_8, 20 ohm
All resistors ½ watt unless otherwise stated.

M_1, 0-1 milliammeters, D.C.
T_1, 6.3 volt 1 amp. filament transformer
S_1, SPST
S_2, DPST
B_1, 22.5 volt (Burgess Type 4156)
B_2, 45 volt (Burgess Type XX30)
V_1, 38 tube
$V_{2,3}$, 929 phototube
R_7, controls zero adjustment
P_8 controls calibrated dial
$L_{1,4}$, RCA 47 miniature lamps

volt C battery which substitutes for the filament transformers in Fig. 1. Approximately fifty hours' continuous usage can be expected from this model without battery replacement, but for most purposes a line-operated model would be considered more suitable.

The operation of the intrument is simple and rapid. For calibration a piece of blank wax is placed in the carrier and inserted into the apparatus. The

calibrated disk is set on zero, and the zero adjustment dial is turned clockwise until the meter gives a reading. The zero adjustment dial is turned back very slowly until the meter falls to zero. The blank wax is removed from the carrier, and a piece of wax with occlusal impressions is inserted for reading. Next, the calibrated dial is turned to the right until the meter moves from zero, and turned back slowly until the meter drops to zero. Readings can be taken to the closest unit. On very low values there is a time lag of about two seconds in reaching the end point (balance point of the two phototubes), and this must be taken into account in the manipulation. Readings of ten units or more are reached in 1/10 second or less.

COMPARISON IN INSTRUMENT READINGS

Performances calculated from readings on wax registrations are compared with peanut performance in Fig. 2. There is satisfactory correspondence between the values.

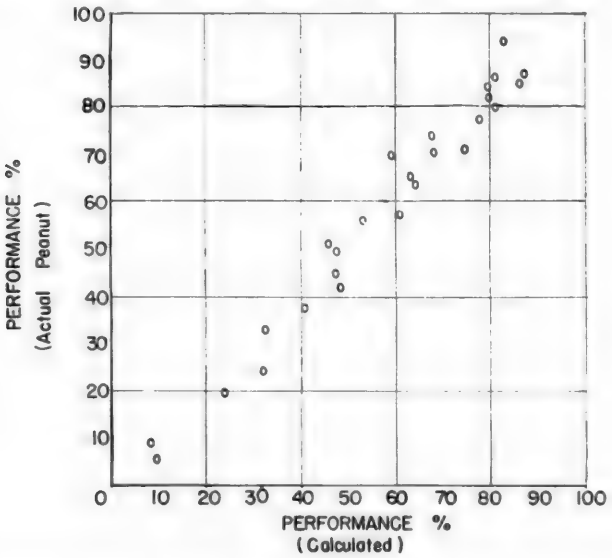

Fig. 2.—Correlation between actual performance by peanut tests and calculated performance read from the modified wax meter.

While the relationship between the original and present model is not completely linear, points follow along a smooth curve, indicating that relative values of readings on the two instruments will be in the same order.

When the readings from the original area meter were compared with the masticatory efficiency,[4] it was found that there was a satisfactory linear relationship between these two variables at the middle range, but that at extremely low area readings or very high area readings the prediction of efficiency from area was likely to be in error. When the cusps of molars were high, area readings were likely to be lower than would be expected from the efficiency of the

dentition, whereas with well-abraded dentition, the area would be higher than would be expected from the masticatory efficiency. Because of the optics involved in this new area meter and the possibility for control of the circuit, the instrument could be designed to make allowance for these discrepancies. By changing the voltage dividers on the phototubes or the amplifier tube, it has been possible to cause waxes to read somewhat higher on extremely low values, about the same in the middle range, and lower on the higher value. A study has been made on twenty-six half dentitions in which readings have been taken of masticatory performance. The correlation coefficient between actual peanut performance and predicted performance as determined by area measurements on the original unit[4] was 0.81, whereas with the modified circuit on the new meter the coefficient was 0.90. The new meter had an advantage in calculating performance, because the performance has been made to be a linear function of area.

SUMMARY

An improved design of food platform area meter has been prepared. The new equipment has many advantages in portability and cost. It possesses the precision of the older instrument, and can be adjusted to eliminate intrinsic errors in the former equipment.

The advice and encouragement of R. S. Manly during this development is acknowledged with appreciation.

REFERENCES

1. Manly, R. S., and Braley, L. C.: Masticatory Performance and Efficiency, *J. D. Res.* **29**: 448-462, 1950.
2. Yurkstas, A., and Manly, R. S.: Measurement of Occlusal Contact Area Effective in Mastication, *Am. J. Orthodontics* **35**: 185, 1949.
3. RCA Phototube Manual Form PT-20R1, 3-48, p. 11.
4. Manly, R. S.: Factors Affecting Masticatory Performance and Efficiency Among Young Adults, *J. D. Res.* **30**: 874-882, 1951.

AGE CHANGES IN THE PERIODONTAL TISSUES OF THE RAT MOLAR

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INTRODUCTION

THE physiologic aging process of the periodontal tissues has been a subject of considerable interest to the dental profession as a whole, and to the periodontist in particular. A knowledge of the physiologic changes of the periodontal tissues with age is essential to an understanding of periodontal diseases.

The purpose of this study was to follow in the rat molar the changes that occur from youth to old age in the position of the epithelial attachment, the incidence and degree of gingival inflammation, the position of the alveolar crest, the state of cellular activity at the alveolar crest, the width of the interdental septum, the position of the cellular cementum, and the amount of root resorption. The rat molar, which is a tooth of limited growth, provides a fair comparison with the permanent human tooth, and has been used for that reason in experimental studies.

An attempt also was made to establish basic information which would be essential to evaluate the results of experimental studies in the molars of rats of different ages.

REVIEW OF THE LITERATURE

Comparatively few studies have been reported on the effects of age on the periodontal structures.

The discovery of the epithelial attachment by Gottlieb (1921) led Orban and Köhler (1924) to determine the position of the epithelial attachment in teeth of thirty-eight jaws of human beings ranging in age from 2 to 50 years. Gottlieb (1922) observed that the molars of rats frequently were loosened or exfoliated by an inflammatory process caused by hairs and strands of grain embedded in the gingivae.

Skillen (1930) studied the development of the epithelial attachment in the rat molar. He used buccolingual sections of about 500 molars of rats ranging in age from 19 days to 3 years. Skillen concluded that the changes from youth to old age occur in the following sequence: (a) the attachment of a primary cuticle to the enamel which develops following atrophy of the ameloblasts; (b) the transformation of the enamel epithelium into the epithelial attachment; (c) the keratinization of the surface of the gingival epithelium; and (d) the

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downgrowth of the epithelial attachment along the cementum in the absence of any visible pathologic condition.

Shepro (1947) noted that gingival inflammation of the rat molar was not a function of the age of the animal. He found that the prevalence and severity of gingival inflammation remained relatively constant throughout all age groups.

Hoffman and Schour (1940) described the appositional pattern of growth of alveolar bone and cementum, using alizarin-injected rats from 14 to 500 days of age. They concluded that apposition of alveolar bone at the crests and fundi, and of secondary cementum, was a continuous process throughout the life of the animal. Vertical eruption, therefore, was also a continuous process to compensate for attrition and growth of bone at the alveolar crests.

Sicher and Weinmann (1944) described the physiologic distal drift of the rat molar teeth in correlation with the growth of the maxilla and mandible. They found that apposition of bone along the mesial alveolar wall and resorption of the bone along the distal wall were characteristic for all the molar teeth. The distal drift was an intermittent process as shown by a cyclic reparative apposition of bone on the resorbed surfaces with an alternating disorganization and reorganization of the periodontal membrane.

Shepro (1947) found that the interdental septum of the rat molars undergoes physiologic resorption that increases in severity with age, and that this resorptive process proceeds from the periodontal membrane surface of the interseptal bone, leading to an atrophy of the interdental septum.

MATERIAL STUDIED

The material used in this study consisted of histologic sections of the lower molars of ninety-five normal rats. The animals constituted part of the Wistar Age Study Program supported by the Fels Fund. The animals were sacrificed at various age intervals from 21 days up to 1,000 days.

The animals consisted of two strains, Wistar strain albino and Norwegian gray rats. The group between 21 and 300 days of age consisted of forty-six albino rats; the group between 600 and 1,000 days consisted of twenty-eight albino and twenty-one gray rats. No sections of the albino strain were available between 300 and 600 days of age, and no sections of the Norwegian gray strain were available under 600 days of age. All the rats were bred under controlled conditions and fed on a diet of Purina Dog Chow pellets, and maintained in a similar environment.

The heads of the animals were bisected anteroposteriorly and the jaws fixed in 5 per cent formalin, decalcified in 5 per cent nitric acid, and embedded in celloidin. Each jaw quadrant was sectioned in a mesiodistal direction, and the sections stained with hematoxylin and eosin. The incisors were not included in this study.

METHODS OF ANALYSIS

Epithelial Attachment.—The position of the epithelial attachment in each periodontal area was tabulated according to one of the four stages of epithelial attachment first described by Orban and Köhler (1924) (Fig. 1).

Stage 1 represents that position wherein the epithelial attachment is confined to the crown of the tooth with its apical end located at the cemento-enamel junction. *Stage 2* represents that position in which the apical end of the epithelial attachment has migrated apically onto the cementum with the coronal end still located on the enamel. *Stage 3* is designated as that position in which the epithelial attachment is located wholly on the cementum with its coronal end, or bottom of the gingival sulcus, located at the cemento-enamel junction. *Stage 4* indicates that position in which the epithelial attachment is located entirely on the cementum with its coronal end, or bottom of the gingival sulcus, located apical to the cemento-enamel junction.

The particular stage of the epithelial attachment was tabulated for the mesial and distal surface of each molar.

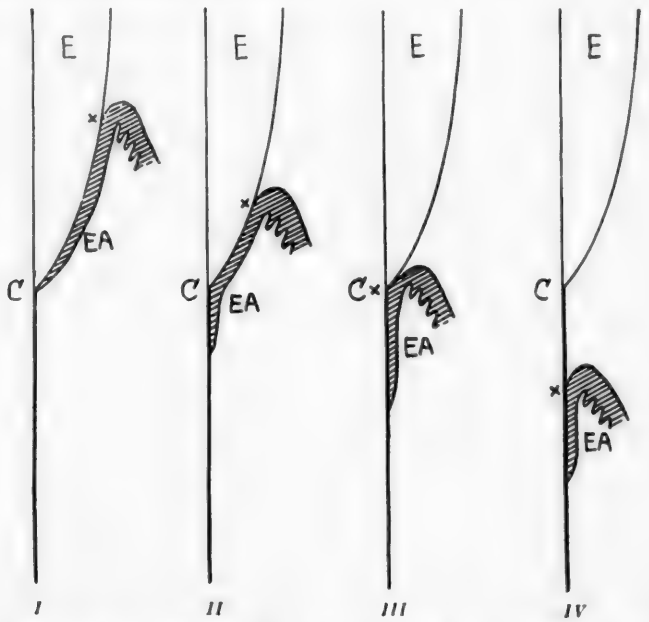

Fig. 1.—Diagrammatic illustration of the four stages of epithelial attachment. *E* = enamel; *EA* = epithelial attachment; *C* = cemento-enamel junction; *X* = bottom of gingival sulcus. (From Orban, Oral Histology and Embryology, 1949, The C. V. Mosby Company.)

Gingival Inflammation.—The degree of gingival inflammation was classified by inspection as none, mild, moderate, or severe in each papillary gingival unit. Typical examples of the four grades of inflammation are illustrated in Fig. 2.

The area was classified as having no gingival inflammation when no more than four or five inflammatory cells could be distinguished, and as mild to severe in accordance with the density of infiltration and the increased numbers of inflammatory cells present. Such a classification is open to criticism because the gradation of the borderline cases depends on subjective judgment. However, though there may be some disagreement in separating mild from mod-

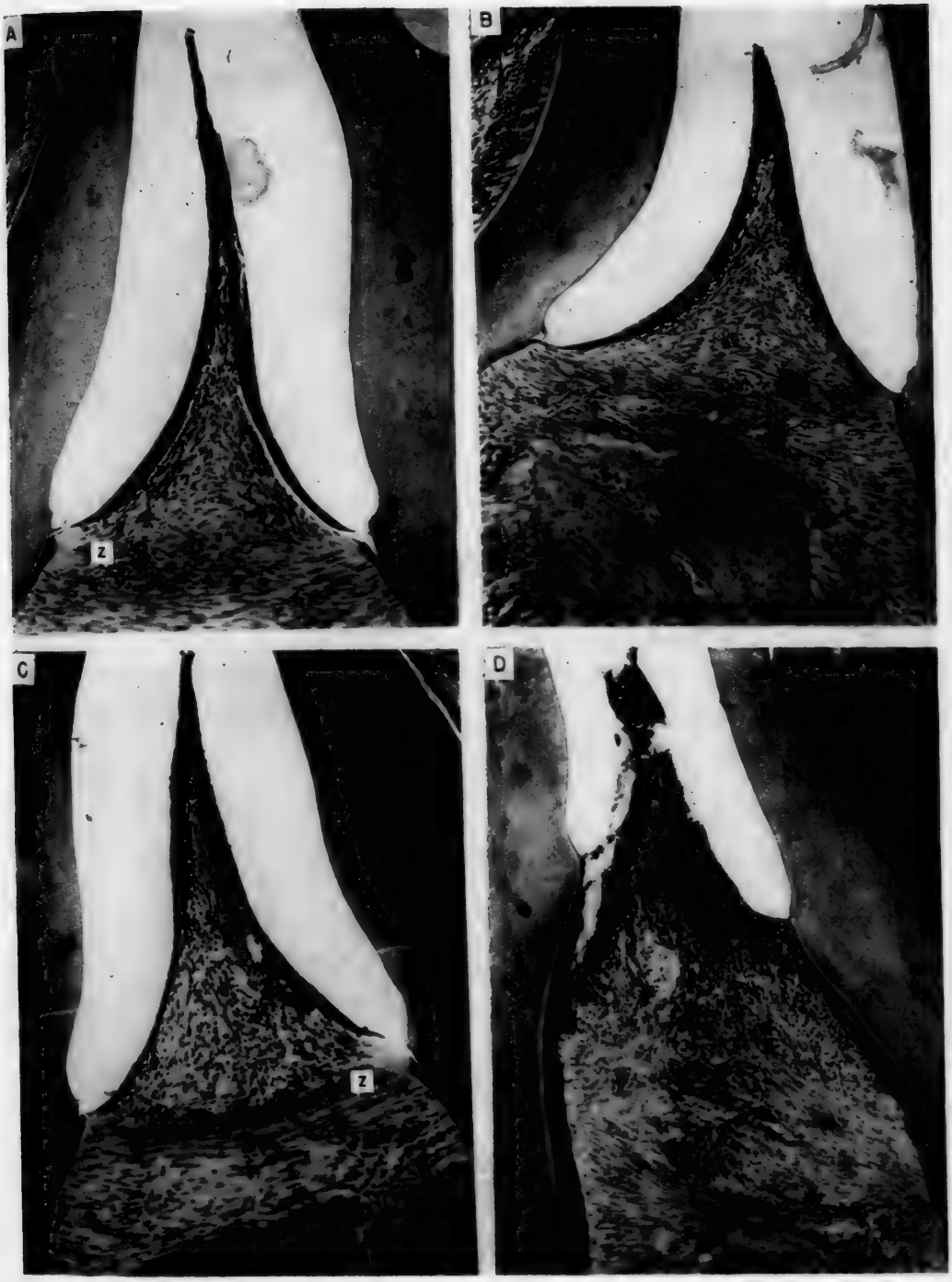

Fig. 2.—Photomicrographs of interdental papillae showing four degrees of gingivitis (×120). Hematoxylin and eosin stain; *A*, no gingivitis, 50 day albino (No. 250); *B*, mild gingivitis, 50 day albino (No. 250); *C*, moderate gingivitis, 50 day albino (No. 321); *D*, severe gingivitis, 700 day albino (No. 406). *A*, *B*, and *C* show epithelial attachment in Stage 1. *D* shows epithelial attachment in Stage 2. *A* and *C* show cementum spur or knob at *z*.

erate, or moderate from severe, the difference between mild and severe is quite obvious.

Position of the Alveolar Crest, Cellular Cementum, and Width of the Interdental Septum.—In an attempt to relate the position of the alveolar crest to the tooth, a fixed anatomic landmark, the cemento-enamel junction, was used as a point of reference. The distance between the highest point of the alveolar crest in mesiodistal sections and cemento-enamel junction was tabulated in microns for the mesial and distal surfaces of each tooth.

In order to estimate what part apposition or resorption of bone played in determining the position of the alveolar crest at any given age, the cellular activity at each crest was classified as being in a state of apposition, rest, or resorption. In addition, attention was paid to the plane of resting lines near the crest of the septum to gain information on the functional relations between tooth and septum.

In order to measure the progressive decrease in width of the alveolar septum, micrometer measurements were made at the cemento-enamel junction of the approximal distance between the first and second, and second and third molars. The progressive decrease in width of the alveolar septum with age is illustrated in Fig. 3.

Measurements were made also of the distance between the cemento-enamel junction and the coronal end of the cellular cementum on the mesial and distal surface of each tooth.

Root Resorption.—The surface of the roots of each tooth was examined for evidence of root resorption, and notation was made of the root surface where the areas of resorption were located.

FINDINGS

Inasmuch as two strains of rats, the Wistar albino and the Norwegian gray, were included in this study, the opportunity was offered to compare the effects of age on the periodontal structures of the two strains. No Norwegian gray specimens were available under 600 days of age so that comparisons be-

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Fig. 3.—Photomicrographs showing progressive changes in interdental tissues of rat from 50 to 1000 days of age (×60). Hematoxylin and eosin stain. *A*, 50 day albino (No. 268); *B*, 300 day albino (No. 26); *C*, 700 day albino (No. 392); *D*, 900 day Norwegian gray (No. 995); *E*, 1,000 day Norwegian gray (No. 1001). Note in *A* at 50 days of age wide interdental septum with apposition of bone at alveolar crest. Note also thin layer of acellular cementum and position of coronal end of cellular cementum. Epithelial attachment is in Stage 1.

Note in *B* at 300 days of age narrower interdental septum with resorption lacunae along its mesial wall, and incremental pattern of bone apposition shown by resting lines at alveolar crest which continue along distal wall of septum. Note also increased thickness of acellular cementum with no change in position of coronal end of cellular cementum. Epithelial attachment is in Stage 1.

Note in *C* at 700 days of age further narrowing of interdental septum with no intervening bone between adjacent roots in area of apex producing a defect in septum. Recent resorption has occurred along distal wall of septum, although older resting lines indicate distoclusal drift of teeth. Note also increased thickness of acellular cementum with no change in position of coronal end of cellular cementum. Cellular cementum at apex has increased root length by one-third from *x* to *xx*. Distance from alveolar crest to cemento-enamel junction is considerably greater than in the younger animal. Note also uncalcified cementum spur or knob at *z*, covered by epithelial attachment. Epithelial attachment is in Stage 2.

Note in *D* at 900 days of age complete resorption of interdental septum caused by a more uniform approximation of two adjacent roots along their entire length. Periodontal fiber attachments now run from cementum to cementum. Note position of coronal end of cellular cementum is farther from cemento-enamel junction in this Norwegian gray animal than in the albino. Note lesser amount of cellular cementum and how surface of the cellular cementum is more sharply outlined than in the albino.

Note in *E* at 1,000 days of age closer approximation of two adjacent roots with root resorption at area of closest approximation. Interdental septum has been entirely resorbed. Epithelial attachment is in Stage 4.

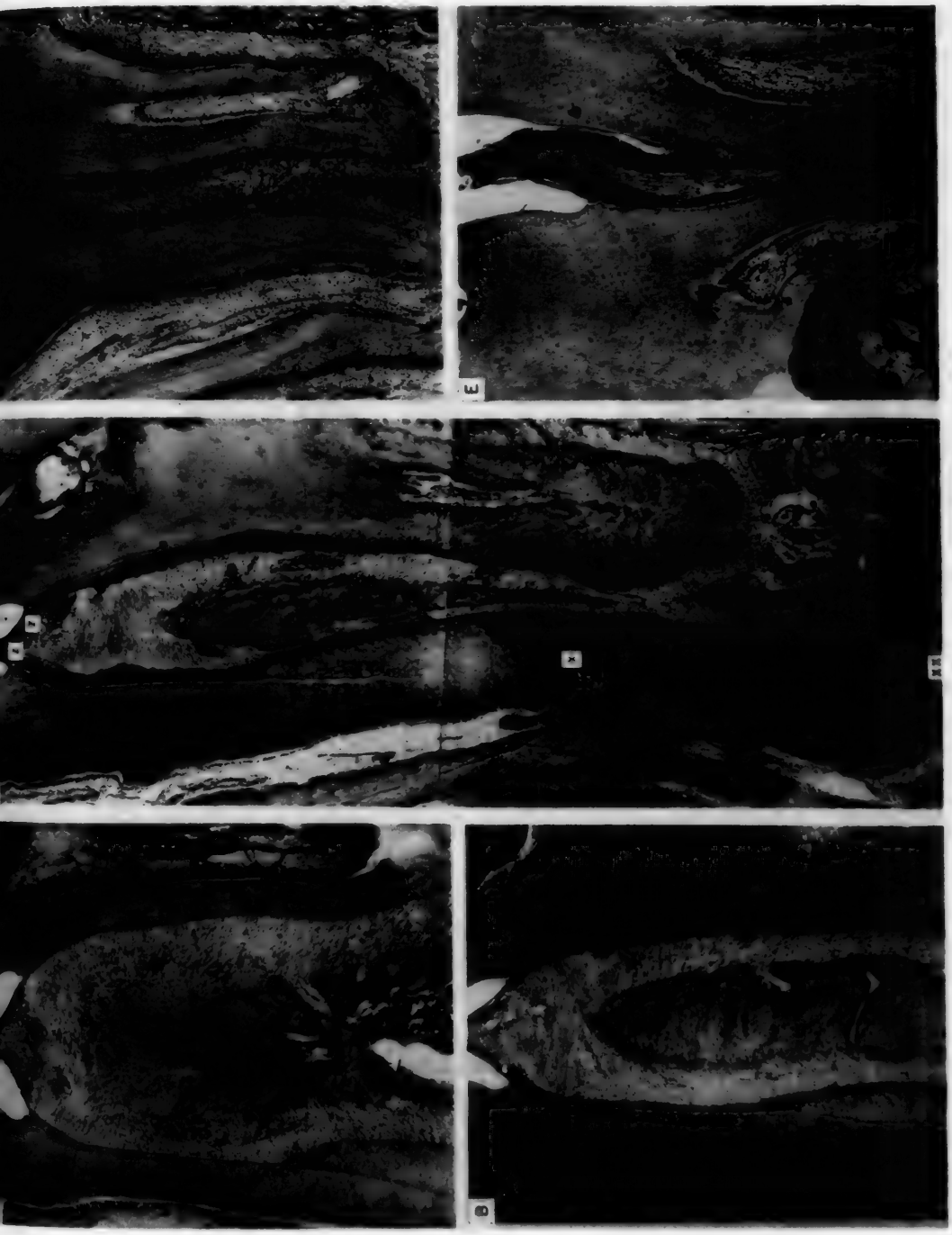

Fig. 3.—(*For legends, see opposite page.*)

tween the two strains were limited to age levels between 600 and 1,000 days of age. A study of the progressive effects of age was possible in the albino group with sections available at successive intervals from 21 to 300 days, and again from 600 to 1,000 days.

In order that a picture of the effect of age on the periodontal structures may be obtained from youth to old age, the data from 21 to 1,000 days for the Wistar albino strain will be presented first, followed by a comparison of the two strains between the 600 to 1,000 day age levels.

Wistar Albino Strain 21 to 1,000 Days of Age

Epithelial Attachment.—In the area mesial to the first molar, the downgrowth of the epithelial attachment started at an earlier age (100 days) and reached Stage 4 at an earlier age (200 days) than in any of the other five

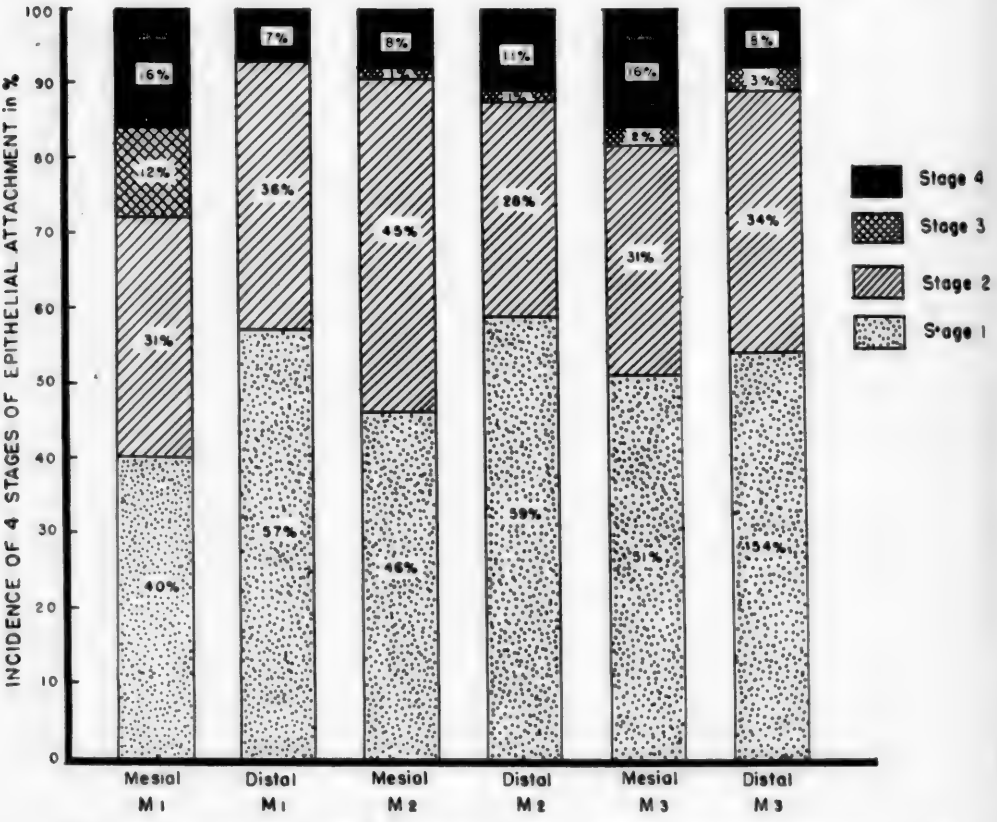

Fig. 4.—Histogram showing distribution of each stage of epithelial attachment in each area for albino strain—21 to 1,000 days of age. Note that downgrowth of epithelial attachment on mesial of each tooth is farther advanced than on distal.

areas. In the area distal to the third molar the start of the downgrowth of the epithelial attachment occurred later (300 days) than in any of the other five areas.

The rate of downgrowth of the epithelial attachment followed a consistent pattern of being greater on the mesial side of each of the three molar teeth than on the distal (Fig. 4).

The epithelial attachment in all cases was found to be restricted to the enamel (Stage 1) in all animals up to 75 days of age. The youngest animal in which part of the epithelial attachment was found on the cementum (Stage 2) was 100 days old. The first cases wherein the entire epithelial attachment was found on the cementum (Stages 3 and 4) occurred at the 200 day age level. Thereafter, a progressively larger percentage of epithelial attachments was seen in Stage 4 (Fig. 5).

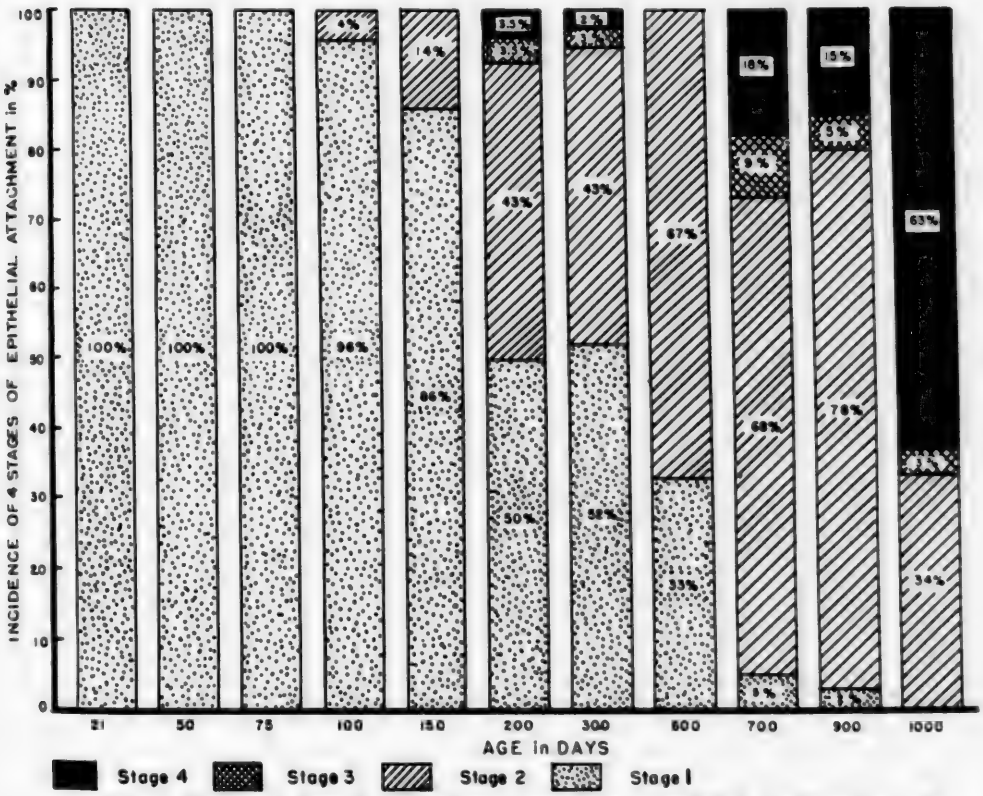

Fig. 5.—Histogram showing distribution of each stage of epithelial attachment for each age group for albino strain—21 to 1,000 days of age. Note trend of farther downgrowth of epithelial attachment as age increases.

Gingival Inflammation.—There was a marked similarity in the incidence of gingivitis in the papillae mesial to the first molar and distal to the third molar. The incidence of gingivitis likewise was similar in the two interdental papillae. However, the incidence of gingival inflammation in the two interdental papillae was markedly greater than in the papillae mesial to the first molar and distal to the third molar (Fig. 6). One would expect such a condition to occur because of the greater likelihood of food impaction in the interdental areas.

In examining the distribution of gingivitis in all areas combined (Fig. 7), an unsteady increase in the severity of gingival inflammation could be seen from youth to old age. It should be noted, however, that this increase was not so much caused by an increase in the incidence of moderate or severe inflammation, but largely by higher percentages of the mild type of gingivitis.

It was noted previously that the downgrowth of the epithelial attachment in the area mesial to the first molar occurred earlier and reached Stage 4 at an earlier age than in any of the other five areas. Yet, the incidence of gin-

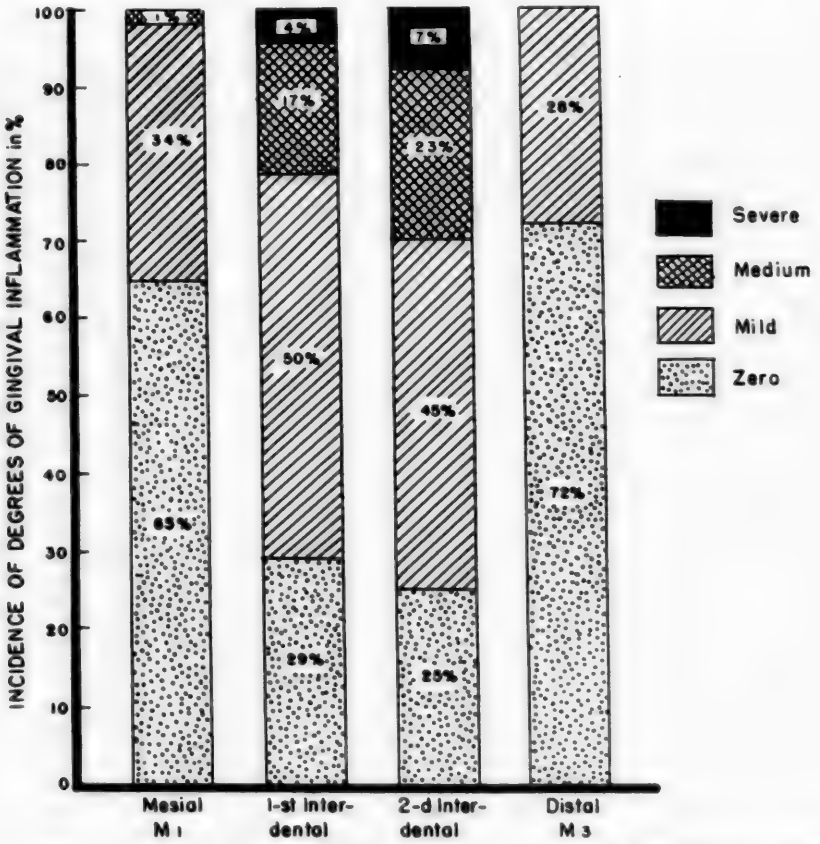

Fig. 6.—Histogram showing incidence of degrees of gingival inflammation in papillae of the albino strain—21 to 1,000 days of age. Note markedly greater incidence and severity of gingivitis in two interdental papillae than in papillae mesial to first molar and distal to third molar.

givitis in this area was markedly less than in the first and second interdental areas. Thus, no relation between the incidence of gingivitis and downgrowth of the epithelial attachment could be ascertained.

Fibrous food and hairs occasionally were seen lodged between the teeth gingival to the contact areas, causing an inflammatory reaction usually graded as severe. This condition was seen more commonly in the older age groups

(Fig. 8). In spite of this occasional food impaction, downgrowth of the epithelial attachment was not as great in the two interdental areas as it was mesial to the first molar. Nor was pocket formation commonly seen in these areas of severe food impaction.

Position of the Alveolar Crest.—Over 350 micrometer measurements of the distance between the alveolar crest and the cemento-enamel junction mesial and distal to each of the three molar teeth were made, and the arithmetic mean values, expressed in microns, were determined for each of the six positions for each age group. The alveolar crest mesial to the first molar was two to three times farther distant from the cemento-enamel junction than any of the other

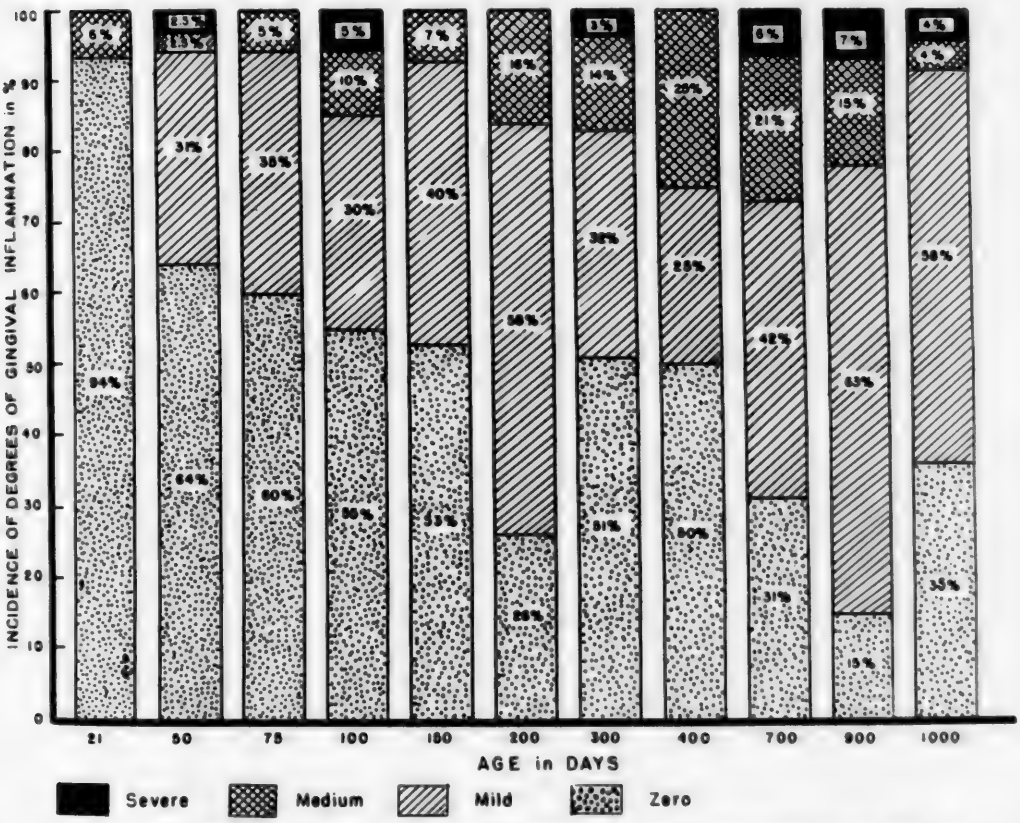

Fig. 7.—Histogram showing incidence of degrees of gingival inflammation for each age group for the albino strain—21 to 1,000 days of age. Note slight increase in incidence and severity of gingivitis with age.

crests in the 50-day-old animal (measurements of the 21-day-old animal were omitted, since the second and third molars at that age were not fully erupted into occlusion). The crest mesial to the first molar, furthermore, maintained this greater distance from the cemento-enamel junction throughout all age groups.

Fig. 8.—Photomicrographs of debris impaction between teeth. Hematoxylin and eosin stain. *A*, 900 day albino, ×400 (No. 390); *B*, 900 day albino, ×120 (No. 390). Note in *A*, at 900 days of age, apposition of bone at alveolar crest in presence of severe inflammation. Note in *B* complete resorption of interdental septum with relatively high location of the epithelial attachment (Stage 4) on root surface in presence of severe irritation.

There was a definite trend for the distance between each alveolar crest and the cemento-enamel junction to increase as age progressed. Each of the six curves illustrating the increase in distance between each alveolar crest and the cemento-enamel junction with age was of the parabolic type, with its steepest inclination in the younger age groups. These curves indicated a slowing of the rate of increase in distance between the alveolar crest and the cemento-enamel junction as age progressed (Fig. 9).

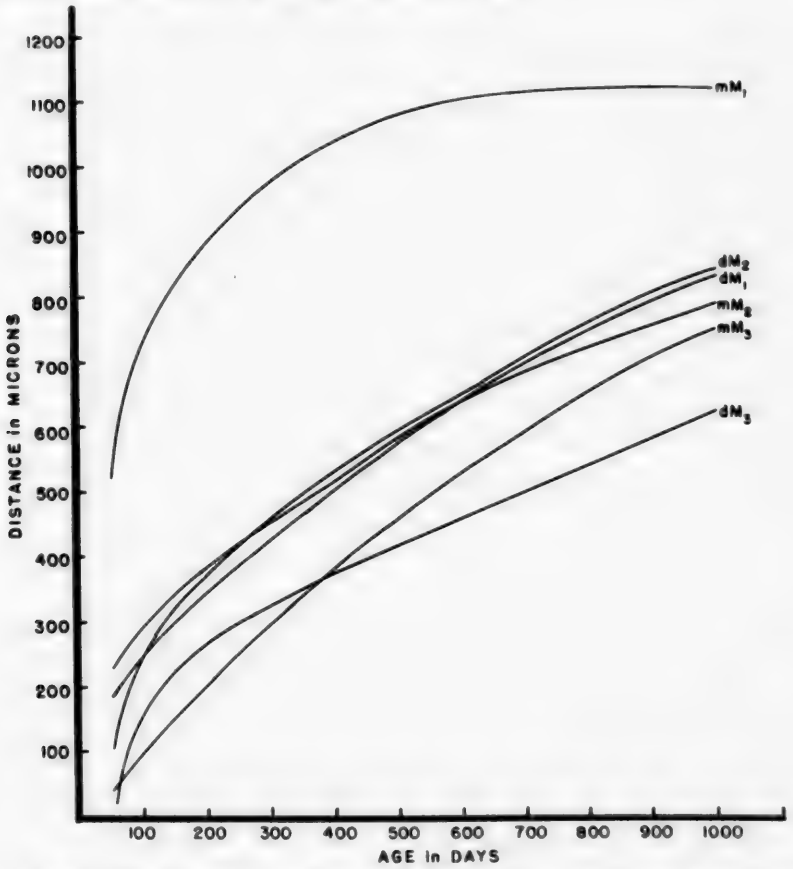

Fig. 9.—Graph showing age gradient of increase in distance between each alveolar crest and cemento-enamel junction. Note steeper inclination of each curve in the younger age groups. *m*, Mesial; *d*, distal; M_1, first molar; M_2, second molar; M_3, third molar.

The increase of the distance of the alveolar crest from the cemento-enamel junction was different on the mesial and distal surface of each tooth. It was found always to be greater on the distal than on the mesial. These findings are but the expression of the increasing mesial tipping of the molars.

State of Cellular Activity at the Alveolar Crest.—Apposition of bone at the alveolar crest was seen from 21 to 900 days of age. However, from 300 to 700 days some instances of arrest of bone apposition could be seen. From 700 to 1,000 days, occasional signs of resorption occurred, indicating some inter-

ruption of growth at the alveolar crest, since apposition was seen in two instances even in the 900-day-old animal (Fig. 8, *A*).

The surface of the septum was sometimes seen slanting distally and apically, but even here, where the height of the crest was too sharp to allow a fixation of the plane of its free surface, resting lines ran in mesiodistal sections from mesially and coronally, distally and apically. The functional part of the septum (alveolar bone proper) was higher on the mesial side of the septum than on its distal surface.

Width of the Interdental Septum.—In correlation to the distal drift of the rat molars, resorption was a very common finding along the mesial wall of the interdental septum, whereas apposition was more commonly seen on the distal wall of the septum. The mesiodistal diameter of the interdental septa showed a gradual decrease with progressive age (Fig. 3, *C*). In several instances in the 900- and 1,000-day-old animals, the interdental septum between the first and second, and second and third molars even showed defects.

These defects were either slits starting at the alveolar crest and reaching a variable distance apically, or round or oval holes somewhere between the base and the free border of the septum. All the defects corresponded to the areas of closest approximation of adjacent roots.

Measurements of the interproximal distances at the cemento-enamel junction for each age group made in an effort to quantitate this approximation of teeth showed a continuous decrease with age. The decrease was about equal in the first and second interdental spaces, and amounted to about 32 per cent of the interdental width of the youngest animal.

In 70 per cent of the cases the interproximal distance between the first and second molars was greater than the interproximal distance between the second and third molars.

Position of the Coronal End of the Cellular Cementum.—Over 300 micrometer measurements of the distance between the coronal end of the cellular cementum and the cemento-enamel junction on the mesial and distal sides of each of the three molar teeth were made.

Analysis of the data indicated that the distance between the coronal end of the cellular cementum and the cemento-enamel junction remained constant after the 50 day age level. These data support the findings of Hoffman and Schour (1940) showing that the growth of the secondary cementum occurs in overlapping increments at the apex, none of the successive increments reaching as far coronally as the one preceding it.

Root Resorption.—The number of areas of root resorption increased unsteadily from youth to old age. There appeared to be a tendency for the distal surfaces of the roots of the first and second molars to show a greater incidence of resorption than the mesial surfaces. Since the number of areas of resorption seen for each root surface in the albino strain was small, the validity of this conclusion is open to question. Weinmann and Schour (1945), however, noted a similar tendency for resorption areas to be localized to the distal root surfaces in the white rat.

Only twenty-six root surfaces showed evidence of resorption out of the entire group of seventy-four animals of the albino strain, indicating that the factors which cause root resorption are relatively slow and intermittent for the albino group.

Cementum Spur.—The presence of a cementum spur or knob at the cemento-enamel junction was noted in many of the albino animals (Figs. 2, *A* and *C*, and 3, *C*). The spur appeared as a localized area of hypercementosis at the coronal end of the acellular cementum. Its staining reaction was eosinophilic, resembling cementoid tissue rather than the characteristic basophilic reaction of calcified cementum.

Norwegian Gray Strain and Comparison of the Findings With Those of the Wistar Albino Rats, 600 to 1,000 Days of Age

In comparing the effects of age on the periodontal structures in the two strains, it was necessary to use a similar number of animals at each age level for each strain in order not to weight the data at any particular age level. With the material available this could only be approximated, since many of the Norwegian gray animals were sacrificed at irregular intervals, whereas almost all of the Wistar albino animals were sacrificed at 100 day intervals. However, an arrangement of the material could be made using twenty-one animals of the Norwegian gray strain with an average age of 813 days, as compared with twenty-eight animals of the Wistar albino strain with an average age of 821 days between the 600 and 1,000 day age levels. This comparison was sufficiently accurate to bring out the salient differences between the two strains.

Epithelial Attachment.—There was no difference between the two strains in the rate of downgrowth of the epithelial attachment in any of the six areas. The combined data for each of the six areas showed a striking similarity between the two strains in the percentage of cases seen in each of the four stages of epithelial attachment.

Gingival Inflammation.—Also, the incidence of gingival inflammation in each of the four areas was similar for the two strains, although the similarity was not so perfect as in the case of the epithelial attachment. The percentage differences, though slight, were in favor of the Norwegian gray strain, having slightly less gingival inflammation than the Wistar albino strain.

The higher incidence of gingivitis in the two interdental spaces, as compared with the areas mesial to the first and distal to the third molars, was as marked in the Norwegian gray strain as in the Wistar albino strain.

Position of the Alveolar Crest, Cellular Cementum, and Width of Interdental Septum.—The distance of the alveolar crest from the cemento-enamel junction was on the average 26 per cent greater in the Norwegian gray than in the albino strain. However, the state of cellular activity at the aveolar crests in the two strains was similar.

The distance of the coronal end of the cellular cementum from the cemento-enamel junction was on the average 34 per cent greater in the Norwegian gray than in the albino strain.

The width of the interdental septum as indicated by the interproximal distance at the cemento-enamel junction was about 16 per cent less in the Norwegian gray than in the albino animal. Furthermore, the interdental septum showed defects in eleven instances in the Norwegian gray animal as compared with only four instances in the albino.

Root Resorption.—Significant differences also were found in the incidence of root resorption in the two strains. Whereas only eighteen root surfaces showed resorption in the twenty-eight animals of the albino strain, fifty-three root surfaces showed resorption in the twenty-one animals of the Norwegian gray strain, a difference in the incidence of resorbed root surfaces of approximately four to one (Fig. 10).

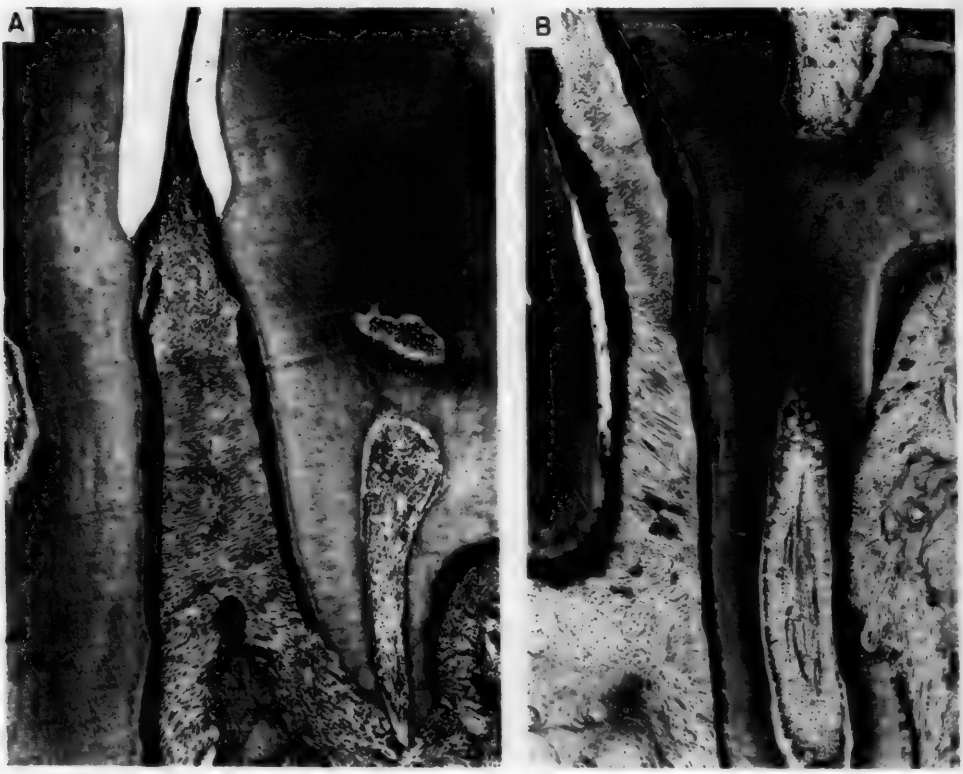

Fig. 10.—Photomicrographs of root resorption (×60). Hematoxylin and eosin stain. *A*, 900 day Norwegian gray (No.994); *B*, 878 day Norwegian gray (No. 22).

In the Norwegian gray animals resorption was seen on the mesial and distal surfaces of all roots, with the exception of the mesial surface of the distal root of the second molar. A majority of the resorption lacunae were in an inactive or repaired state. The highest incidence of resorption, however, was seen in the two interdental areas, where the roots of the first and second, and second and third molars approximated each other, with the higher incidence seen in the second interdental area.

Character of Cementum.—An additional difference between the two strains was noted in the character and morphology of the cementum. The outer surface of the cementum of the albino strain was very rough and spinous in appearance. The apices of the spinous processes blended into the principal periodontal fibers so that a sharp line of demarcation could not be seen between the cementum and the collagenous fibers of the periodontal membrane. By contrast, the outer surface of the cellular cementum of the Norwegian gray animal appeared relatively smooth, was well demarcated from the surrounding connective tissue of the periodontal membrane by a resting line, and in general had an appearance not unlike that of human cellular cementum (Fig. 3, *D*).

Also it appeared that a greater amount of cellular cementum was present in the albino than in the Norwegian gray strain at similar age levels. The cementum spur seen at the cemento-enamel junction in the albino strain was not seen in the Norwegian gray.

The differences noted in the amount, character, and morphology of the cementum of the two strains indicated greater activity of the cementoblasts in the albino strain. The greater activity of the cementoblasts of the albino animal might explain the lower incidence of root resorption seen in these animals.

DISCUSSION

Correlation of Downgrowth of the Epithelial Attachment and Incidence of Gingival Inflammation.—Analysis of the findings for the epithelial attachment and gingival inflammation and a correlation of the two factors showed that the downgrowth of the epithelial attachment was unassociated with the degree of gingival inflammation (Table I). The pattern of downgrowth of the epithelial attachment in each of the four areas seemed to be correlated to the age of the tooth. This confirms early observations by Gottlieb (1921). In the area mesial to the first molar, one of the areas where the incidence of gingival inflammation was lowest, the downgrowth of the epithelial attachment was most marked. In the first and second interdental spaces, where gingival inflammation was markedly greater than in the area mesial to the first molar, the degree of downgrowth of the epithelial attachment was considerably less.

TABLE I

SUMMARY OF AGE CHANGES IN THE PERIODONTAL TISSUES OF THE ALBINO RAT MOLAR

	50 DAYS	200 DAYS	700 DAYS	1,000 DAYS
Predominant stage of epithelial attachment	Stage 1	Stage 1	Stage 2	Stage 4
Predominant degree of gingival inflammation	None	Mild	Mild	Mild
Average distance of alveolar crest to cemento-enamel junction	182 microns	501 microns	618 microns	907 microns
Predominant type of cellular activity at alveolar crest	Apposition	Apposition	Arrest of apposition	Resorption
Average interproximal distance at cemento-enamel junction	413 microns	225 microns	161 microns	278 microns
Average distance of cellular cementum to cemento-enamel junction	952 microns	1017 microns	1036 microns	944 microns

The reason for the earlier downgrowth of the epithelial attachment mesial to each tooth compared to the distal is not clear at this time.

Pocket formation in the rat was not commonly seen, even in cases of severe food impaction between the teeth. Nor were pockets often seen in those cases where defects were present in the interdental septa. These observations confirm the finding and conclusions of Hoffman and Schour (1940) who stated that, "The rat molar presents ideal physiologic conditions in masticatory function and adjustments which are not common in the human teeth."

The frequency of periodontal diseases, even loss of teeth, observed by Gottlieb (1922) and explained by him as a consequence of impaction of hairs and strands of grain, was in all probability due to a substandard diet. In animals fed on a balanced diet of Purina Dog Chow pellets, the gingival conditions were different from those observed by Gottlieb.

Position of the Alveolar Crest.—Apposition was by far the commonest finding at the alveolar crest (Table I). Therefore, the increase in distance with age from alveolar crest to cemento-enamel junction was probably due to the fact that the rate of eruption of the teeth was faster than the rate of appositional growth at the alveolar crest. This finding is clearly in support of previous findings of Hoffman and Schour (1940), who have noted that the rate of growth of cementum and bone at the fundus was faster than appositional growth of bone at the alveolar crest. Resorption at the alveolar crest was observed only occasionally in the old animals. In fact, apposition was present even in some cases of severe inflammation (Fig. 8, *A*).

The increase in distance between the alveolar crest and the cemento-enamel junction followed a gradient, indicating a slowing of the growth and eruptive process as age increased. This type of age gradient supports the eruption and growth rates of the rat molar described by Hoffman and Schour (1940).

The increase in distance with age between the highest point of the alveolar crest and the cemento-enamel junction was greater on the distal than on the mesial of each tooth. This finding is a manifestation of the mesial tipping of the three molar teeth as age advanced. The distal half of the occlusal surfaces of the teeth appeared to show greater attrition than the mesial half in the older animals. This is another indication of the mesial tipping of the teeth with age.

The fact that the alveolar crest mesial to the first molar was two to three times more distant from the cemento-enamel junction than any of the other crests, and maintained this greater distance throughout all age groups, is believed to be an anatomic peculiarity associated with the large diastema between the first molar and the incisor (Fig. 9).

The obliquity of the resting lines in the crest of the interdental septum has to be interpreted as a correlation of the septal growth pattern to the tipping of the teeth during progressive eruption. In consequence of the mesial tipping, the cemento-enamel junction of the anterior tooth is found at a higher level than that of the posterior tooth. The appositional pattern of the septum makes its mesial half grow faster than the distal half. Thus, the functional relation between each tooth and the corresponding part of the interdental septum is maintained.

Width of the Interdental Septum.—The decrease in the interproximal distance at the cemento-enamel junction from the youngest to the oldest animal (Table I) is due to interproximal wear at the contact areas, therefore causing the approximation of the mesial and distal roots of adjacent teeth.

In areas of defects of the interdental septum, the principal fibers of the two periodontal membranes took on the appearance and function of interdental fibers, with their attachment at either end in the cementum of the adjacent roots.

Difference of the Two Strains in the Position of the Alveolar Crest, Cellular Cementum, and Width of the Interdental Septum.—The greater distances between the alveolar crest and the cemento-enamel junction noted in the Norwegian gray animals, as compared with the albino, are believed to be due to the larger size of the animal and of its teeth.

The greater distances between the coronal end of the cellular cementum and the cemento-enamel junction noted in the Norwegian gray animals mainly are due to the larger size of the teeth. Also, a difference in amount of cementum apposition between the two strains could be ascertained. Cementum apposition in the Norwegian gray seemed to be slower than in the albino animal.

The greater decrease in width of the interdental septum with age found in the Norwegian gray strain indicates a greater intensity of interproximal attrition. Barber (1949), in a microscopic investigation of the pattern of occlusal attrition in the rat molar, also found significantly greater occlusal attrition in the Norwegian gray animal than in the albino. Occlusal attrition and interproximal attrition, of course, are interdependent factors.

Based on the finding of greater interproximal attrition, an explanation of the greater loss of interdental septum seen in the Norwegian gray strain can be made. The result was a closer approximation of adjacent roots along their entire length, with the concomitant greater loss of interdental bone seen in the Norwegian gray strain. In some cases the divergence of the roots of the rat molar from the central axis of the tooth resulted in approximation of adjacent roots in the apical area first as interproximal wear occurred, restricting the defect of the interseptal bone to the area of nearest approximation (Fig. 11).

From previous investigations (Hoffman and Schour, 1940, and Sicher and Weinmann, 1944) and the findings of this study, the migratory movements of the rat molar teeth from 21 to 1,000 days of age may be summarized as follows: While continuing to erupt in an occlusal direction throughout the life of the animal, the molar teeth not only tilt mesially while migrating distally (Fig. 11), but they tilt lingually while shifting slightly in a buccal direction.

Difference in the Amount of Root Resorption Seen in the Two Strains.—The markedly greater severity of root resorption seen in the Norwegian gray strain is correlated with two factors: (a) greater interproximal attrition resulting in closer approximation of adjacent roots, and (b) greater occlusal trauma associated with greater occlusal attrition.

The root surfaces showing the highest incidence of resorption were the distal surfaces of the distal roots of the first and second molars, and the mesial surfaces of the mesial roots of the second and third molars. These are the

root surfaces facing one another in the two interdental areas. Greater interproximal attrition permitted a closer approximation of the adjacent roots, with a concomitant greater decrease in width of the interdental septum. The incidence of root resorption in these areas, therefore, was directly correlated with the intra-alveolar resorption of the interdental septum.

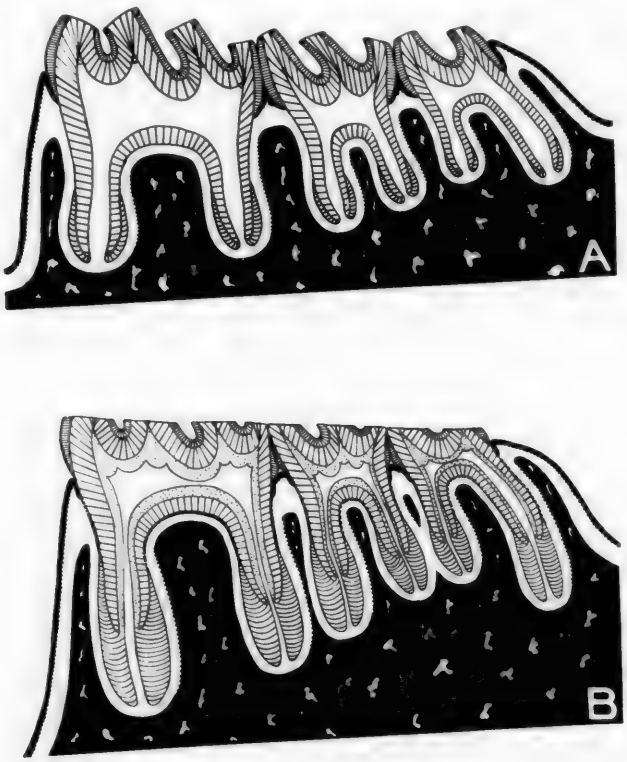

Fig. 11.—Semidiagram showing age changes of epithelial attachment, dentin, and alveolar bone in mandibular molars of the albino rat; *A* at 50 days of age and *B* at 1,000 days of age. Note: (1) amount of occlusal and interproximal attrition, (2) epithelial attachments at Stage 1 in *A* and at Stage 4 in *B*, (3) amount of secondary dentin, (4) amount of cellular cementum and position of coronal end of cellular cementum, (5) amount of acellular cementum, (6) width of interdental alveolar septa, (7) distance of alveolar crest to cemento-enamel junction, and (8) resorption on mesial aspects of interdental and interradicular septa.

All other areas of the roots of the Norwegian gray strain also showed a greater incidence of resorption. This finding may be attributed to greater occlusal trauma associated with the greater occlusal attrition already noted and to less cementoblastic activity. The molar teeth of the Norwegian gray animal apparently are subjected to greater forces during normal masticatory function.

The relationship of the epithelial remnants in the periodontal membrane to the biology of cementum long has been a subject of conjecture. It is interesting to note in this connection that Wentz (1950), using the same animals that were used in this investigation, found that the Norwegian gray rats

showed a greater prevalence of specimens with high epithelial remnant counts than did the albino rats. Orban and Weinmann (1942) have noted proliferation of epithelial remnants in the periodontal membrane of human teeth in several cases diagnosed as periodontosis. Schour (1934) also noted an increase in the number and size of epithelial rests in the periodontal membrane of the molars of hypophysectomized rats. He also found less than the normal amount of cementum in the molars in animals with a greater than normal amount of resorption. Whether proliferation of the epithelial remnants occurs as part of an adaptive mechanism by which cementum production is stimulated or whether their very presence inhibits cementum production are questions which await further evaluation. It is interesting to note that in the two strains of rats compared in this study, there is a parallelism between the numbers of areas of root resorption and the numbers of epithelial remnants.

Cementum Spur.—The observation of an uncalcified cementum spur in the dog, pig, and squirrel led Gottlieb (1943 and 1944) to believe that uncalcified cementum acts as a barrier to downgrowth of the epithelial attachment. However, the epithelial attachment was seen covering the eosinophilic cementum spur in several of the rat molars of the albino strain (Fig. 3, *C*), indicating that the epithelial attachment can grow over uncalcified cementum. The formation of the spur may, therefore, be interpreted as a localized reaction of the cementum to adjacent gingival inflammation. Absence of the spur in the Norwegian gray animals, however, is one more indication of less cementoblastic activity in the Norwegian gray strain.

SUMMARY

This quantitative study of the age changes in the supporting tissues of the rat molar was based on histologic examination of seventy-four normal Wistar albino and twenty-one Norwegian gray rats 21 to 1,000 days of age.

Tabulation of the data included the level of the epithelial attachment, degree of gingival inflammation, position of the alveolar crest in relation to the cemento-enamel junction, state of cellular activity, and pattern of apposition at the alveolar crest, width of the interdental septum, distance of the cellular cementum from the cemento-enamel junction, and amount of root resorption.

The findings may be summarized as follows:

Age Changes in the Wistar Albino Strain 21 to 1,000 Days of Age

1. Downgrowth of the epithelial attachment progressed as the age of the animal increased, so that at 21 days of age 100 per cent of the epithelial attachments were found only on the enamel (Stage 1), and at 1,000 days of age 75 per cent of the epithelial attachments were found only on the cementum (Stage 4).

2. Downgrowth of the epithelial attachment occurred earlier and progressed farther on the mesial of each molar tooth than on the distal.

3. The incidence of gingival inflammation was lower in the gingival papillae mesial to the first molar and distal to the third molar than in the papillae located between the molar teeth.

4. The incidence of papillary gingivitis tended to increase with age.

5. The distance between the alveolar crest and the cemento-enamel junction increased with age. The rate of increase in distance between these two points diminished as age advanced, indicating a slowing of the growth and eruption process with age.

6. Bone apposition occurred at the alveolar crest between 21 and 900 days of age. Bone resorption occasionally was seen at the alveolar crest in the very old animals.

7. The appositional pattern at the crest of the interdental septa can be ascertained from the resting lines. They slant distally and apically. Thus, the height of the functional part of the septum serving tooth attachment is greater on the mesial than on the distal.

8. Width of the interdental septum between the most prominent parts of the approximating roots decreased from 50 to 1,000 days of age, about 32 per cent by resorption, largely along its mesial wall, causing slitlike defects, or a perforation of the septum.

9. The position of the coronal end of the cellular cementum did not change between 50 and 1,000 days of age.

10. Root resorption occurred most commonly on approximating root surfaces of adjacent teeth, and the number of areas of resorption tended to increase as the age of the animal increased.

Difference Between the Wistar Albino and Norwegian Gray Strains 600 to 1,000 Days of Age

1. The alveolar crest in the Norwegian gray animal is about 28 per cent more distant from the cemento-enamel junction than in the albino.

2. The coronal end of the cellular cementum in the Norwegian gray animal is about 34 per cent farther from the cemento-enamel junction than in the albino.

3. The Norwegian gray strain shows a greater decrease with age in the interproximal distance at the cemento-enamel junction than the albino.

4. Incidence of root resorption is approximately four times greater in the Norwegian gray strain than in the albino.

5. There is a greater amount of cementum in the albino than in the Norwegian gray strain.

6. An uncalcified cementum spur at the cemento-enamel junction was seen in the albino, and not in the Norwegian gray strain.

7. Downgrowth of the epithelial attachment, the incidence of gingival inflammation, and the state of cellular activity at the alveolar crest are similar in both strains.

CONCLUSIONS

1. Downgrowth of the epithelial attachment is a physiologic process related to the age of the animal, but unrelated to the presence or absence of moderate gingival inflammation.

2. The presence of an uncalcified cementum does not act as a barrier to downgrowth of the epithelial attachment.

3. The increase between the alveolar crest and the cemento-enamel junction with age is due to a faster rate of eruption than the rate of appositional growth at the alveolar crest.

4. The defects in the interdental septum are a result of interproximal wear at the contact areas.

5. There is a greater interproximal attrition in the Norwegian gray strain than in the albino.

6. Differences noted in the amount, character, and morphology of the cementum of the two strains indicate less cementoblastic activity in the Norwegian gray strain than in the albino.

7. The significant differences noted in the two strains of animals used in this study indicate the importance in experimental work of giving consideration to possible inherent differences in various strains of the same species.

REFERENCES

Barber, T. K.: Relation of the Physical Character of the Diet to the Pattern of Attrition and Caries Experience in the Molars of the Albino Rat, Thesis, University of Illinois, 1949.

Barber, T. K.: Personal communication.

Gottlieb, B.: Der Epithelansatz am Zähne, *Deutsche Monatschr. f. Zahnh.* **39**: 142, 1921.

Gottlieb, B.: Die Paradentalpyorrhöe der Rattenmolaren, *Vschrift. Zahnh.* **38**: 273, 1922.

Gottlieb, B.: Some Cementum Problems, *J. Periodont.* **15**: 77, 1944.

Gottlieb, B.: Continuous Deposition of Cementum, *J. A. D. A.* **30**: 842, 1943.

Hoffman, M. M., and Schour, I.: Quantitative Studies in the Development of the Rat Molar: II. Alveolar Bone, Cementum and Eruption, *Am. J. Orthodont. & Oral Surg.* **26**: 854, 1940.

Orban, B., and Köhler, J.: Die Physiologische Zahnfleischtasche, Epithelansatz und Epitheltieferwucherung (The Physiologic Gingival Sulcus), *Ztschr. f. Stomatol.* **22**: 353, 1924.

Orban, B., and Weinmann, J. P.: Diffuse Atrophy of the Alveolar Bone, *J. Periodont.* **13**: 31, 1942.

Shepro, M. J.: Age Changes in the Periodontal Structures of the Rat Molar, Thesis, University of Illinois, 1947.

Shepro, M. J., Weinmann, J. P., and Schour, I.: Physiologic Atrophy of the Interdental Septum in Rats, *J. D. Res.* **27**: 743, 1948.

Schour, I.: The Hypophysis and the Teeth. III. Changes in the Rat Molar Following Hypophysectomy, *Angle Orthodont.* **4**: 3, 1934.

Sicher, H., and Weinmann, J. P.: Bone Growth and Physiologic Tooth Movement, *Am. J. Orthodontics & Oral Surg.* **30**: 109, 1944.

Skillen, W. G.: The Morphology of the Gingivae of the Rat Molar, *J. A. D. A.* **17**: 645, 1930.

Weinmann, J. P., and Schour, I.: Experimental Studies in Calcification. I. The Effect of Rachitogenic Diet on the Tissues of the White Rat, *Am. J. Path.* **21**: 821, 1945.

Wentz, F. M., Weinmann, J. P., and Schour, I.: The Prevalence, Distribution and Morphologic Changes of the Epithelial Remnants in the Molar Region of the Rat, *J. D. Res.* **29**: 637, 1950.

THE VOLUMETRIC CONTRACTION OF DENTAL GYPSUM MATERIALS ON SETTING

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OF ALL the materials associated with dental practice and technic, those containing gypsum products are probably used most extensively. In keeping with this fact, a great deal of research has been directed toward problems involving the physical behavior of these materials. It is felt, however, that knowledge is lacking on some of the more fundamental behavior characteristics. The manifestation of this deficiency is the presence of many phenomena which exist when dental gypsums are used. An illustrative example would be the hygroscopic expansion of dental casting investments containing gypsum as a binder. Since there is no established theory on the mechanism of this action, it is just as difficult for the dental manufacturer to compound an adequate material as it is for the dentist to use it properly. The purpose of this paper is to resolve some of these difficulties by studying one very fundamental characteristic of these materials, namely, the volumetric behavior of dental gypsums during the process of setting.

All dental gypsum products which include plaster, stone or hydrocal, the improved stones, and the binder of most gold casting investments are essentially composed of the hemihydrate form of calcium sulfate ($CaSO_4 \cdot \frac{1}{2}H_2O$). When mixed with water, the hemihydrate is converted to the dihydrate form of calcium sulfate ($CaSO_4 \cdot 2H_2O$) and this process is synonymous with the observed setting action. Specifically associated with this reaction is the paradox which exists with respect to the volumetric behavior of the mixed mass. It is common knowledge that an expansion accompanies the setting process; yet, a contraction should occur based on theoretical considerations as is shown in Table 1. Skinner[1] wrote of this anomaly and explained that the expansion is only apparent and is possibly caused by the thrusting of the gypsum crystals in the direction of their growth during setting. It is the specific purpose of this paper to determine whether a volumetric contraction does exist and, if so, how it can be explained in the presence of a measurable expansion.

A thorough search of the dental and chemical literature was of no assistance except for one article by J. H. Van't Hoff[2] in which the author measured a contraction of plaster of Paris during setting. However, the method of measurement as well as the objectives of the experiment precluded any adaptation of the results to the problem at hand. Accordingly, an apparatus was designed and tests were run to measure the true volumetric behavior of dental gypsum materials on setting.

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TABLE I
THEORETICAL CONTRACTION BASED ON SPECIFIC GRAVITY VALUES

Chemical reaction	$CaSO_4 \cdot \frac{1}{2}H_2O$	+	$3/2H_2O$	→	$CaSO_4 \cdot 2H_2O$
Molecular weights	145.148		1.5 × 18.016		172.172
Specific gravities	2.75		.997		2.32
Volume	$\frac{145.148}{2.75}$	+	$\frac{27.024}{.997}$	→	$\frac{172.172}{2.32}$
	52.78	+	27.10	→	74.21

$$\text{Volumetric contraction} = \frac{(52.78 + 27.10) - 74.21}{52.78 + 27.10} \times 100 = 7.10\%$$

DESCRIPTION OF THE APPARATUS AND TEST METHOD

Fig. 1 shows the apparatus used, along with a schematic diagram indicating its component parts. The setup operates on a dilatometer principle in which the sample of plaster, stone, or investment is placed in a closed fluid system and the change in volume is determined by the movement of the fluid in a capillary tube.

Immediately after being mixed, the material is poured into an inlay ring having a base on which a wire hook has been soldered. Another long wire hook was used to lift the ring and place it carefully into the flask (*D*). The glass cap (*C*) was placed onto the flask (*D*), a seal being achieved by virtue of a ground glass joint. Another ground glass joint seals the capillary tube into the glass cap (*C*). The fluid used in this system was water, which was stored in reservoir (*A*) and allowed to enter the system through valve (*B*). Thermocouples in series were placed in the fluid system to allow for compensation of exothermic and ambient temperature effects on the fluid system.

With a moderate degree of speed, fiducial measurements could be made 4 to 6 minutes after the start of mix. Volumetric behavior previous to this time was determined by extrapolation.

The capillary tube was calibrated and found to hold .007 c.c. of water per lineal centimeter. Since readings could be taken to within 1 mm., the accuracy of measurement was .0007 c.c. or percentagewise (using 25.7 c.c. as the initial volume) approximately .003 per cent change in volume could be recorded. When the water column in the capillary tube approached the bottom of the scale, valve (*B*) was opened, the water column rose to the top of the scale, and further readings were taken which were then added to the previous readings, thereby yielding a continuous recording.

The use of water as the ambient fluid in this system might appear to influence the measurements on the basis of the solubility of the hemihydrate in the mixed mass of plaster, stone, or investment; however, tests were run using a saturated solution of the hemihydrate as the ambient fluid and no appreciable differences were recorded.

It is particularly relevant to note that this apparatus measures only true volumetric changes and that apparent volumetric changes are not reflected in the volume change of the fluid system. It is believed that what is commonly

known as setting expansion (due possibly to a thrusting of the gypsum crystals in their growth) is an apparent volumetric change, and although it perceptibly increases the outer dimensions of the sample, the true volume has not been affected. For this particular situation, therefore, no volume change would be registered.

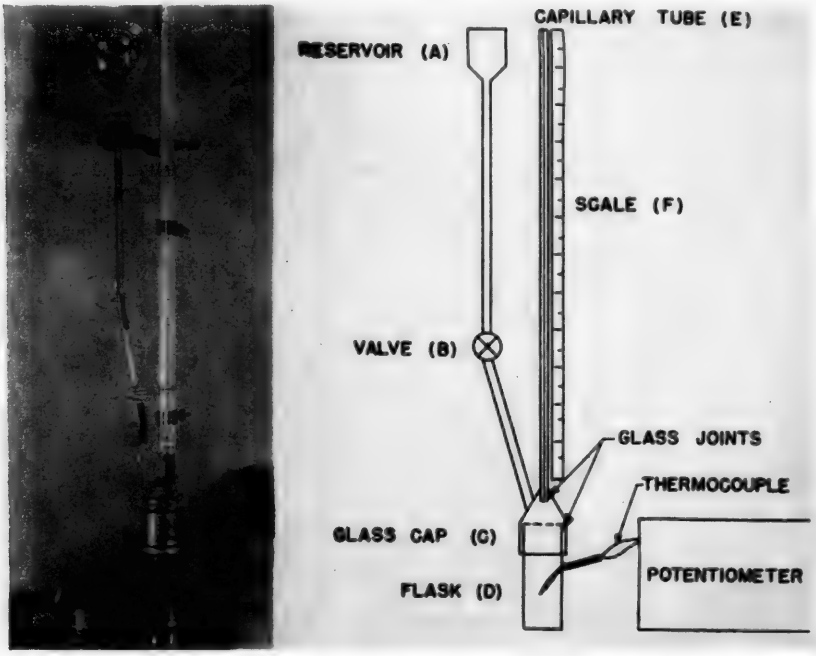

Fig. 1.—Apparatus used to determine the volumetric contraction of gypsum materials.

RESULTS AND DISCUSSION

In *Fig. 2* is shown typical curves for the true volumetric behavior of the same initial volume of plaster, stone, improved stone, and a hygroscopic casting investment. As can be seen, a volumetric contraction accompanies the setting process. From these tests, it is apparent that although the outer dimensions of the sample increase slightly, on the basis of setting expansion measurements, the true volume of the mass has decreased. This decrease must be manifest in a porosity formation within the sample, which is about equal in volume to the measured contraction. On the basis of this porosity formation, a driving force must then exist within the setting mass which draws in the ambient atmosphere, whether it be air, water, or any other gaseous or fluid medium.

Several other points of information can be determined by a study of Fig. 2: (1) The slope of these curves at any particular point is simply the rate of reaction in the process of conversion of the hemihydrate to the dihydrate form of calcium sulfate. This may prove to be of further research value when the setting process itself is more thoroughly investigated. (2) The completeness

of the reaction at any time after mix can be established. As can be seen from the curves, the major portion of the reaction has been completed at 30 minutes and at 1 hour to 1½ hours, the reaction has been almost entirely completed. This substantiates a point which one of us (D. B. M.)[3] raised previously in which the completion of the reaction on the basis of hardness tests was observed to be approximately 1 hour after mix. In Fig. 3 is shown two curves for a gypsum material which is used as a binder in a hygroscopic casting investment. The setting time of the material has not been controlled, the first run experiencing an initial Gilmore setting time of 9½ minutes and the second,

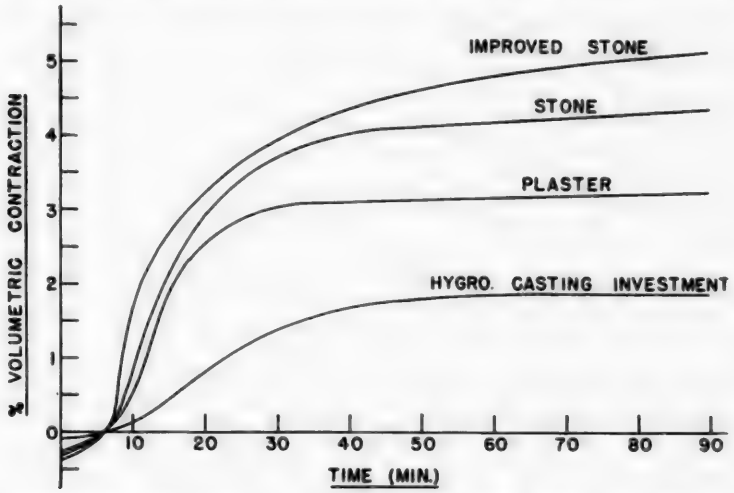

Fig. 2.—Volumetric contraction (c.c.) of the various dental gypsum materials in per cent of the original volume (c.c.) encompassed by the inlay ring.

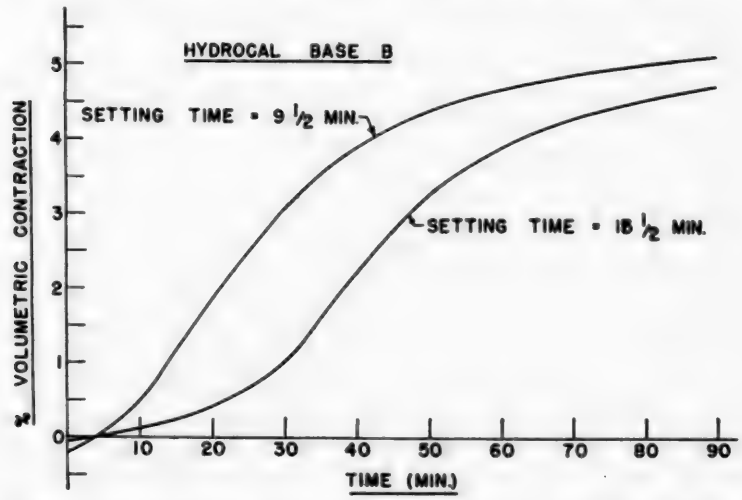

Fig. 3.—Volumetric contraction (c.c.) of the binder used in a hygroscopic casting investment in per cent of the original volume (c.c.) encompassed by the inlay ring.

a setting time of 18½ minutes. Because of this situation, the significance of these volumetric contraction curves can be illustrated more clearly. The longer setting time run has a relatively slower rate of reaction and completes any specific portion of its reaction at a later time than the shorter setting time run; however, both reactions tend toward completion at about the same time and they observe the same total contraction value after the completion of the reaction, irrespective of the paths they choose to follow. (3) The amount of the ambient atmosphere to be drawn into the sample during the setting process, for the same initial volume of mix, varies with respect to the type of material being used (Fig. 2). The improved stone draws in the most, dental stone next, then plaster, and finally the casting investment. The reason for this is the difference in the amount of hemihydrate present in the volume encompassed by the inlay ring for the different materials. This in turn is due to the different water/powder ratios used and the difference in the amount of hemihydrate present in the original powders. When these conditions are accounted for, the volume contraction of the different materials is about the same, within the accuracy of determination, as will be shown directly.

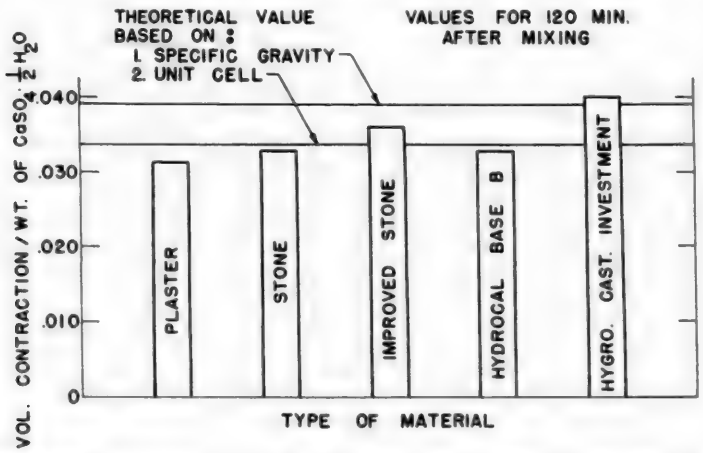

Fig. 4.—Volumetric contraction (c.c.) of the various dental gypsum materials per weight of $CaSO_4 \cdot \frac{1}{2}H_2O$ (Gm.) in the original volume encompassed by the inlay ring.

To analyze these curves more thoroughly and to validate the cause of this phenomenon, a bar graph is presented in Fig. 4 in which the total contraction at 120 minutes after mixing is given for all the materials tested in addition to the contraction which should occur theoretically; however, instead of representing equal initial total volumes encompassed by the inlay ring as is shown in Fig. 2, this graph is the contraction per unit weight of the hemihydrate present in these initial volumes. Theoretically, since the contraction is caused by the conversion of the hemihydrate plus water to the dihydrate, the amount of contraction measured should depend on the amount of hemihydrate present in the inlay ring. The bar graph in Fig. 4 is a result of this postulation. The information and calculations necessary for the values of Fig. 4 are given in

Tables II, III, IV, V, VI, and VII, and are listed as follows: Table II, chemical analysis of the gypsum materials studied; Table III, weight of the powder plus mix water in the original volume and the water/powder ratio of mix; Table IV, calculation of the weight of hemihydrate in the original volume; Table V, unit cell measurements and volumes; Table VI, calculation of theoretical contraction per weight of hemihydrate present; and Table VII, calculation of experimental contraction per weight of hemihydrate present.

TABLE II
CHEMICAL ANALYSIS OF GYPSUM MATERIALS STUDIED
(BASED ON GALLO-WINTERBOTTOM[4] ANALYTICAL METHODS)

CONSTITUENT	PLASTER	STONE	IMPROVED STONE	HYDROCAL BASE B	HYGROSCOPIC CASTING INVEST-MENT
$CaSO_4 \cdot \frac{1}{2}H_2O$	85.0%	87.0%	85.5%	86.0%	26.0%
Soluble anhydride	2.5	.8	1.7	2.5	3.3
$CaSO_4 \cdot 2H_2O$	5.0	3.7	3.3	5.2	5.7
Overburnt anhydride and impurities	7.5	8.5	9.5	6.3	

Effective percentage of $CaSO_4 \cdot \frac{1}{2}H_2O$ includes soluble anhydride, since this material is extremely hygroscopic and readily converts to the hemihydrate form.

TABLE III
WEIGHT OF GYPSUM MATERIALS STUDIED AFTER MIXING AND FOR THE VOLUME OF THE INLAY RING USED
(WATER/POWDER RATIOS OF MIX ARE ALSO GIVEN)

MATERIAL	WEIGHT (GRAMS)	WATER/POWDER RATIO
Plaster	51.0	50/100
Stone	58.5	30/100
Improved stone	62.0	23/100
Hydrocal base B	59.3	26/100
Hygroscopic casting investment	57.5	30/100

Water/powder ratios were determined by a consistency test similar to ADA spec. No. 2 for dental inlay casting investments.

TABLE IV
WEIGHT OF $CaSO_4 \cdot \frac{1}{2}H_2O$ IN THE GYPSUM MATERIALS STUDIED FOR THE VOLUME OF THE INLAY RING USED

MATERIAL	TOTAL WEIGHT (TABLE III)		WEIGHT OF POWDER PER TOTAL WEIGHT (TABLE III)		WEIGHT OF $CaSO_4 \cdot \frac{1}{2}H_2O$ PER WEIGHT OF POWDER (TABLE II)		WEIGHT OF $CaSO_4 \cdot \frac{1}{2}H_2O$
Plaster	51.0	×	$\frac{100}{150}$	×	.875	=	29.8 Gm.
Stone	58.5	×	$\frac{100}{130}$	×	.878	=	39.5 Gm.
Improved stone	62.0	×	$\frac{100}{123}$	×	.872	=	44.0 Gm.
Hydrocal base B	59.3	×	$\frac{100}{126}$	×	.885	=	41.7 Gm.
Hygroscopic casting investment	57.5	×	$\frac{100}{130}$	×	.293	=	13.0 Gm.

The over-all conclusion to be drawn from Fig. 4 is that the values of the measured contraction are quantitatively within the range of the theoretically determined contraction, thereby validating the cause of this volumetric behavior.

TABLE V
UNIT CELL MEASUREMENTS AND VOLUMES*

MATERIAL	CRYSTAL FORM	A	B	C	β	Z
$CaSO_4 \cdot \frac{1}{2}H_2O$	Monoclinic	11.94	6.83	12.70	89°24′	12
$CaSO_4 \cdot 2H_2O$	Monoclinic	5.67	15.15	6.51	118°23′	4

$$V_{CaSO_4 \cdot 2H_2O} = \frac{11.94 \times 6.83 \times 12.70 \times \sin 89°24'}{10^8 \times 10^8 \times 10^8} = 1036 \times 10^{-24}$$

$$V_{CaSO_4 \cdot \frac{1}{2}H_2O} = \frac{5.67 \times 15.15 \times 6.51 \times \sin 118°23'}{10^8 \times 10^8 \times 10^8} = 492 \times 10^{-24}$$

*There is some difference of opinion on the crystal form of the hemihydrate. The values in this table were taken from Gallitelli,[5] who appears to have done the most work on this subject.

TABLE VI
THEORETICAL VOLUMETRIC CONTRACTION PER WEIGHT OF HEMIHYDRATE ON THE BASIS OF SPECIFIC GRAVITY VALUES AND UNIT CELL VALUES

Specific Gravity (x = weight of hemihydrate)

$$\frac{x}{145.148} \times \frac{145.148}{2.75} + \frac{x}{145.148} \times \frac{1.5 \times 18.016}{.997} \rightarrow \frac{x}{145.148} \times \frac{172.172}{2.32}$$

$$\Delta V = \frac{x}{145.148} (52.78 + 27.10 - 74.21) = .039x$$

$$\frac{\Delta V}{x} = .039 \text{ c.c./Gm.}$$

Unit Cell

$$\frac{x}{145.148} \times \frac{6.06 \times 10^{23} \times 1036 \times 10^{-24}}{12} + \frac{x}{145.148} \times \frac{1.5 \times 18.016}{.997} \rightarrow \frac{x}{145.148} \times \frac{6.06 \times 10^{23} \times 492 \times 10^{-24}}{4}$$

$$\Delta V = \frac{x}{145.148} (52.32 + 27.10 - 74.54) = .034x$$

$$\frac{\Delta V}{x} = .034 \text{ c.c./Gm.}$$

TABLE VII
EXPERIMENTAL VOLUMETRIC CONTRACTION PER WEIGHT OF HEMIHYDRATE PRESENT IN THE INLAY RING VOLUME

Plaster	$\frac{\Delta V}{x} = \frac{.93}{29.8}$	=	.031 c.c./Gm.
Stone	$\frac{\Delta V}{x} = \frac{1.29}{39.5}$	=	.033 c.c./Gm.
Improved stone	$\frac{\Delta V}{x} = \frac{1.58}{44.0}$	=	.036 c.c./Gm.
Hydrocal base B	$\frac{\Delta V}{x} = \frac{1.36}{41.7}$	=	.033 c.c./Gm.
Hygroscopic casting investment	$\frac{\Delta V}{x} = \frac{.52}{13.0}$	=	.040 c.c./Gm.

No attempt will be made at this time to annalyze either the difference in values of the various materials or their respective deviations from the theoretical value. This is due to the following observations which may influence the true values of either the experimental or theoretical contraction:

1. The theoretical value of contraction is for a complete reaction which may be difficult to achieve under the conditions of mixing which were used. Also, the experimental values were taken at 120 minutes, at which time some of the contraction curves still indicate a slight positive slope. Therefore, additional conversion of the hemihydrate to the dihydrate can conceivably occur after 120 minutes.

2. It is felt that the theoretical value of contraction based on specific gravity values is not as accurate as that based on unit cell measurements; however, at the present time, there is no unanimity of opinion on the type of crystal structure of the hemihydrate form of calcium sulfate in the chemical literature. Consequently, even the contraction based on unit cell measurements can conceivably be slightly in error.

CONCLUSIONS

1. The true volumetric behavior of dental gypsum materials on setting was shown to be a contraction.

2. In view of the observed outer dimensional increase of these materials on setting (commonly known as setting expansion), the physical situation which exists must be a porosity formation within the sample.

3. For a given volume of the mixed mass, the ability of the materials to contract or draw in the ambient atmosphere increases in the following order: hygroscopic casting investment, plaster, stone, hydrocal base B, and improved stone.

4. The measured contraction validates the theoretical change which should occur during the conversion of the hemihydrate form of calcium sulfate plus water to the dihydrate form.

5. The curves obtained by this measurement of contraction indicate the rate of reaction and the completeness of the reaction at any specific time after mixing.

Because of the fundamental nature of this study, it was not deemed necessary to test different manufacturers' products. The results are valid for any material containing the hemihydrate of calcium sulfate. Those materials which have been tested were considered to be representative of the gypsum products used in dentistry.

REFERENCES

1. Skinner, E. W.: The Science of Dental Materials, ed. 3, Philadelphia, 1947, W. B. Saunders Company, p. 36.
2. Van't Hoff, J. H., Armstrong, E. E., Hiurichsen, W., Weigert, F., and Just, G.: *Ztschr. f. phys. Chemie*, Leipz. & Berlin, p. 272, 1903.
3. Mahler, D. B.: *J. Pros. Dent.* **1**: 188, 1951.
4. Gallo, G.: *J. Soc. Chem. Indust.* 694, 1914 (abstract of Italian original).
5. Gallitelli, P., and Büssem, W.: *Periodic Mineral* **4**: 1, 1933.

PERIODIC PRECIPITATION OF CALCIUM SALTS IN VARIOUS ORGANIC SUBSTANCES

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THE most important problem in dental research, aside from caries, has been the etiology of periodontal diseases.

Both ends of the fibers of the supporting apparatus of the tooth are embedded in bony tissues (alveolar bone and cementum). Analysis of conditions in the periodontium points to a biochemical process specific to these tissues, the deposition of calcium salts in the organic ground substance, which should be investigated in a systematic clarification of the etiologic factors in periodontosis, especially since this process is impaired to a greater or lesser degree in all forms of periodontal disease.

In an attempt to clarify the pathogenic factors, the first step must be identification of the components in the process of calcium deposition. Although there is no doubt that organic matter participates in the formation of hard substance, the manner of this participation and the particular constituents of the organic matter involved in the deposition of calcium salts are unknown. We have, therefore, undertaken to investigate this problem, and have clarified the influence on the deposition of inorganic matter in colloidal systems of some constituents of the organic substance.

These experiments were adapted to the physiologic conditions found on the root surface of the tooth where, according to accepted theories, calcium salts are continuously deposited. The physiologic conditions are increased phosphate content of tissue fluid resulting from increased local phosphatase activity, and the presence of a thin layer of organic substance (cementoid) through which the inorganic substance diffuses before being deposited on the cementum.

To conform with these conditions, experiments were carried out as follows: The organic matter of cementoid, which is 30 per cent collagen, was replaced by a 15 per cent gelatin solution, which is closely akin to the collagen. On this was poured a solution of 2N $Na_2H(PO_4)$, which is analogous to the high phosphate ion content of the tissue fluid at the boundary between periodontal membrane and cementoid. If this 2N $Na_2H(PO_4)$ solution is allowed to diffuse into the gelatin, the calcium phosphate formed is not uniformly distributed, but is deposited in a series of bands separated by more or less clear portions of gelatin, producing Liesegang's rings.

It was found that the appearance of Liesegang's rings is a function of variables involved in the deposition of inorganic matter in organic substance. The influence of the following factors on the calcium phosphate ring deposition in gelatin was investigated: the colloid state of the deposition medium, the calcium concentration, and the addition of various biochemical substances.

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RESULTS

The following factors affect the periodic precipitation of calcium salts in organic substances:

I. *The Colloid State of the Deposition Medium.*—It was found that Liesegang's rings do not form either in agar or in mucilage (gummi arabic). The polysaccharides apparently do not provide an adequate milieu for a banded precipitation of calcium salts. In gelatin the conditions are more favorable. It appears, however, that with regard to ring-forming capacity, there are two types of gelatin: in one (gelatin "A") Liesegang's rings develop (Fig. 1, *1*), whereas in the other (gelatin "B") instead of ring formation, a diffuse granular precipitation of the inorganic matter appears (Fig. 1, *7*).

II. *Calcium Concentration.*—The variation in ring-producing capacity of the two gelatins does not depend on the quantity of calcium which they contain. In the ring-producing gelatin "A" the calcium content of the gelatin may be as low as 5.7 mg. per cent, while the calcium content of gelatin "B" may be as high as 75 mg. per cent. When the chemical composition of gelatin "B" is changed by the addition of certain biochemical substances, a ring structure similar to that of gelatin "A" is formed. It appears, therefore, that the concentration of calcium in the organic medium has no decisive influence on the formation of the ring structure, and points, rather, to the importance of the chemical composition of the organic medium in the formation of the ring structure.

III. *The Influence of Various Biologic Substances.*—The biologic substances investigated may be divided into two groups: (A) the lipoids: lecithin, cholesterin, and vitamin D; (B) amino acids: aspartic acid, arginine, ornithine, lysine, serine, and glycine.

A. *The influence of the lipoids: lecithin, cholesterin and vitamin D on the ring formation (Fig. 1):*

Lecithin: The formation of ring structure is markedly facilitated by lecithin. Experimental conditions closely simulated conditions found in the course of the deposition of cementum on the root surface characterized by a solid phase (the cementum), a supersaturated solution of calcium phosphate, and the consequent deposition of calcium phosphate when the fluid comes in contact with the solid phase. In the experiment these conditions were obtained as follows: In a gelatin of known calcium content, containing 40 mg. per cent lecithin, part of the calcium was bound by an adequate amount of 2N $Na_2H(PO_4)$ solution and the resulting small granular calcium phosphate precipitate provided calcium, the solid phase. The solution in contact with this precipitate is saturated with calcium phosphate. A certain amount of calcium in the gelatin remains unaffected by the added phosphate solution. As the 2N $Na_2H(PO_4)$ solution is poured on the gelatin, further calcium phosphate molecules are formed, and the solution in contact with the precipitate becomes supersaturated. The precipitation which results when the supersaturated fluid comes in contact with the solid phase proceeds in an orderly way. The favor-

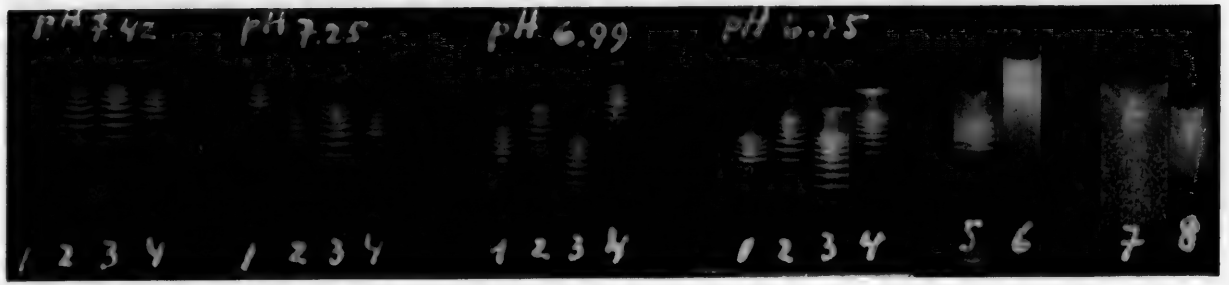

Fig. 1.—The influence of lipoids: lecithin, cholesterin, vitamin D, and pH on ring formation. Gelatin 15 per cent, lecithin content 20 mg. per cent, cholesterin content 800 mg. per cent in *6*, and 200 mg. per cent in *8*. Vitamin D content 30 I.U. per 2 c.c. gelatin. *1*, Gelatin A; *2*, gelatin A + lecithin; *3*, gelatin A + lecithin + vitamin D; *4*, gelatin A + vitamin D; *5*, gelatin A; *6*, gelatin A + cholesterin; 7, gelatin B; *8*, gelatin A + cholesterin.

Fig. 2.—The influence of amino acids on ring formation. *1* = 15 per cent Gelatin B. To this gelatin amino acids are added as follows: *2*, Arginine; *3*, lysine; *4*, arginine, lysine, serine; *5*, aspartic acid; *6*, aspartic acid, serine; 7, aspartic acid, glycine; *8*, aspartic acid, arginine; *9*, aspartic acid, lysine; *10*, aspartic acid, ornithine (*5* to *10* low concentration); *11*, aspartic acid, arginine; *12*, aspartic acid, lysine (high concentration); 13, lecithin (40 mg. per cent in Gelatin *B*).

able effect of the lecithin on the formation of the ring structure is obvious as exemplified by an increase in the number of strata (Fig. 1, *2*).

The effect of cholesterin: In its effect on ring formation, cholesterin antagonizes lecithin. Whereas lecithin facilitates this process at both physiologic and higher concentrations, the influence of cholesterin depends on its concentration in the gelatin. In the presence of 200 mg. per cent (about the cholesterin level in serum) no influence on the formation of ring structure is found, whereas at 800 mg. per cent the strata-forming effect of lecithin is retarded, or even suppressed (Fig. 1, *6*).

The effect of vitamin D: Vitamin D, in contrast to cholesterin, enhances the effect of lecithin on ring formation. The effect of vitamin D is independent on the pH of the medium when combined with lecithin: the number of strata is uniformly increased (Fig. 1, *3*). However, it depends on the pH of the medium when added separately to the gelatin. At pH 7.25, the effect of vitamin D on ring formation is minimal, and the lowest strata are found. At a more acid, as well as a more alkaline pH, formation of ring structure is enhanced by vitamin D. At pH 6.75 and 6.99, as well as at 7.42, the number of strata increases (Fig. 1, *4*). The pH does, however, appear to influence the time required for the ring formation to take place. The ring structure appears more quickly in the acid range, while in the alkaline range the ring structure appears more slowly than at pH 7.25.

The influence of vitamin D on ring formation would indicate that vitamin D exerts a local effect at the zone of the deposition of calcium and phosphorus in the organic substance in the organism.

B. *The influence of amino acids on ring formation (Fig. 2):* The participation of amino acids in the deposition of inorganic matter is very specific. In regard to their effect on the formation of strata, the amino acids examined may be divided into two groups: (1) the dibasic amino acids (arginine, ornithine, lysine, lysine and serine, leucine, tryrosine, and glycine), and (2) aspartic acid.

Each amino acid added to the gelatin brings about a picture characteristic of its own individual group, but unlike that of the ring structure (Fig. 2, *2, 3, 4,* and *5,* respectively). If aspartic acid is added to the gelatin in combination with any one of the amino acids just mentioned, the ring structure will appear (Fig. 2, *11* and *12*).

Of the dibasic acids used in combination with aspartic acid, the best results were achieved with arginine and ornithine. A low concentration of these pairs (aspartic acid 4.5 mg. with arginine 6.0 mg., or with ornithine 5.0 mg. in 2 c.c. gelatin) strikingly facilitates the formation of strata (Fig. 2, *8* and *10*). At higher concentrations, however, the exceptional effect of these amino acid pairs does not appear. Thus, each member of the dibasic acid group used with aspartic acid brings about ring formation in a similar way. In the strata-forming effect, these pairs of amino acids can be replaced by lecithin (Fig. 2, *13*).

DISCUSSION

Of the clinical implications involved in these results the following should be mentioned: Cholesterin, in a concentration higher than physiologic, sup-

presses the favorable effect of lecithin on the formation of ring structure. This specific antagonism between lecithin and cholesterin may be paralleled by their behavior in the organism. The disturbance of cementum deposition in cementopathia is accompanied by a cholesterinemia. It should be pointed out that other diseases which are associated with loosening of the teeth are accompanied by a cholesterinemia, such as diabetes and hypothyroidism. The relation between these conditions and the loosening of the teeth may be accounted for by the cholesterin effect brought about in the process of deposition of inorganic matter.

It was shown that the influence of vitamin D on ring formation depends upon the pH of the medium. I should like to point out, especially, the retarded action of vitamin D on ring formation in an alkaline medium, as it is believed that some disturbance of the acid-base balance of the body, by which the tissue fluids become more alkaline than normal, may be one factor in the disturbance of cementum deposition met with in cementopathia.

CONCLUSIONS

A periodic ring structure, similar to Liesegang's rings, develops in a 15 per cent solution of gelatin when 2N $Na_2H(PO_4)$ solution is poured over and allowed to permeate the gelatin.

The following factors influence this process of rhythmic strata formation: *lecithin,* which facilitates strata formation and produces a ring structure; *vitamin D,* which promotes strata formation and enhances the effect of lecithin; *cholesterin,* which retards strata formation and, in higher concentrations, suppresses the influence of lecithin; *aspartic acid,* which in combination with arginine, ornithine and serine (in order of their efficiency) produces a ring structure.

In agar the formation of ring structure does not take place.

These factors, whose influence on the formation of a periodic ring structure has been presented, may be operative in the organism, as the deposition of cementum on the root surface is essentially the same process that has been investigated, i.e., the deposition of calcium salts within a collagenous medium.

These factors should be considered in further investigations into the question of the continuous deposition of cementum.

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OBSERVATIONS ON THE SALIVARY MUCOLYTIC ENZYMES

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INTRODUCTION

DENTAL caries is believed to be due principally to the action of acid produced from fermentable carbohydrate and, as the salivary mucoids provide a possible source of fermentable carbohydrate, any enzyme system depolymerizing this substrate is of interest.

Simmons[1] found evidence for the existence of an enzyme system with mucolytic activity in saliva, probably derived from the parotid secretion, and Rogers[2] showed that there are organisms in saliva capable of depolymerizing the mucoid. Meyer[3] found that when submaxillary mucoid was incubated with filtered mixed saliva in the presence of toluene, reducing sugar was liberated. Gore[4] claimed that amylase was responsible for this mucolytic action, but his evidence was only circumstantial and in the present investigation purified salivary α-amylase has been shown to have no effect.

Before a study of the action of the mucolytic enzyme system on its substrate could be undertaken, it was necessary to study the variation in enzyme concentration in saliva, the occurrence of mucolytic microorganisms, and the preparation and properties of the enzyme. These preliminary investigations are reported here, and the action of the enzyme preparation on salivary mucoid is described in another paper.[5]

MATERIALS AND METHODS

Three substrates for the mucolytic enzymes have been used, viz., ovomucin,[6] mucoid prepared from saliva,[7] and heat-sterilized saliva. Heating at 80° C. for thirty minutes was found to be effective in sterilizing the great majority of samples of saliva. The heated saliva was centrifuged aseptically, the supernatant withdrawn and tested for sterility, and the sterile samples were pooled.

Various enzyme preparations were incubated with these three substrates, and in every case the enzyme preparation showed the same degree of activity against all the mucoid preparations. Enzymatic activity was measured by three methods: (1) decrease in ACRA (Congo red in acid alcohol) titer,[8] (2) decrease in precipitable mucoid, and (3) decrease in viscosity.

1. Burnet[8] developed a quick method (ACRA method) for determining the mucoid content, depending on the precipitability of mucoids in an acid-alcohol mixture. The decrease in ACRA titer on incubation with salivary

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mucinase was determined. The mucoid preparation is diluted to 3 ACRA doses (ACRA titer greater than 2 but less than 4) and one volume is added to serial twofold dilutions of the enzyme in 0.9 per cent NaCl. After incubation at 37° C. for one hour, one drop of 0.25 per cent Congo red is added to each tube, and the greatest dilution of enzyme which has decreased the ACRA titer to 0 is a measure of the enzyme concentration. If this dilution is 1:8, the enzyme is said to have a titer of 8; if the end point is between 1:8 and 1:16, the titer is 8^+.

2. The second method depends on the precipitability of salivary mucoid by trichloracetic acid (T.C.A.). T.C.A. precipitates most of the salivary mucoid,[9] but after depolymerization of the specimen the addition of T.C.A. produces only a turbidity. The carbohydrate content of whole saliva (filtered through muslin) and of the supernatant after T.C.A. precipitation was estimated by the method of Dische,[10] as modified by Kabat and Mayer.[11] The difference between the total carbohydrate and that in the T.C.A. supernatant is the amount present in the precipitate, and is presumed to be a component of the mucoid. On incubation of saliva, it was found that this difference bears a direct relationship in individual samples to the ACRA titer, being low when the ACRA titer is low, and vice versa. This indicates that approximately the same mucoid fraction is assessed by the ACRA method as is present in the T.C.A. precipitate.

3. Enzymic activity has also been measured by determining the decrease in relative viscosity of individual salivas at 25° C. There is only a slight difference in results obtained for decrease in relative viscosity at 25° C. and 37° C. and, therefore, results obtained for decrease in viscosity at 25° C. can be compared with results for decrease in ACRA titer at 37° C. The decrease in ACRA titer on incubation is accompanied by a decrease in viscosity. Thus, it is possible to compare the results obtained by all three methods. The first method, which depends on the decrease in ACRA titer, has been used for determining the relative activity of all enzyme preparations and samples of saliva, but the second method has also been used where indicated.

EXPERIMENTAL

A. Salivary Enzymes

1. *Variation in Mucolytic Activity.*—Except where otherwise stated, activated saliva was used and was incubated for eighteen to twenty-four hours. This period was necessary because, although initially the relative viscosity of saliva decreases rapidly on incubation, in most cases it does not decrease sufficiently for estimation to be completed on the same day. It was noted that there was a wide variation in the mucolytic activity, as determined by the ACRA method, from less than 1 up to 16, and the variation which could occur in the one person and from person to person was investigated.

(a) Variation with time of incubation: The enzyme titer decreased on prolonged incubation and was 0 after fourteen to twenty-eight days.

(b) Resting and activated saliva: From the one subject a series of samples of resting saliva was collected during the day, on the hour, and a series of

samples of activated saliva was collected on the half hour. There was no significant difference in enzyme activity between resting and activated salivas.

(c) Variation during the day: A series of samples of activated saliva was collected hourly between 10 A.M. and 5 P.M. from the one subject. The enzyme titer varied between 8 and 16, but there was no observable trend during the day.

(d) Variation from day to day: Samples of activated saliva were collected from eighteen people on five different days. The titers varied between less than 1 and 16; the titer 2^+ was most common. The pH was estimated before and after incubation, but there was no relationship between pH or change in pH and enzymic activity. In only one-third of the subjects was the variation in enzyme titer greater than could be due to the variation during the day.

(e) Variation from person to person: The results obtained and analyzed for variation from day to day were also examined for variation from person to person. Arbitrary divisions of the range of titers were made, depending on the variation in titer for the one person (Table I). It is apparent from the table that there are two clearly distinguishable groups, one with a range of 1^+ to 2^+ (I), and the other with a range of 2^+ to 16 (III). Between these is a third, intermediate, group (II). The results, therefore, indicate that there is some variation from person to person.

TABLE I

VARIATION IN ENZYME TITER FROM PERSON TO PERSON

GROUP	LOWEST TITER FOR EACH PERSON	HIGHEST TITER FOR EACH PERSON	NUMBER OF PEOPLE
I	1^+	2^+	5
II	1 - 2	4^+ - 8^+	5
III	2^+	4 - 8	4
	2^+ - 4^+	8^+ - 16	4

(f) Enzyme titer and buffer capacity: The buffering capacity of saliva has been suggested as a method for determination of caries susceptibility.[12, 13] This property was measured, but no relationship could be found between buffering capacity and mucinase titer of saliva.

2. *Purification of Salivary Mucinase.*—Purification of salivary mucinase by precipitation and adsorption was studied. The enzyme titer of most samples of saliva was low, and higher activity was desirable when attempting to purify the enzyme. Ultrafiltration of saliva through a gradocol membrane proved an effective method.

Acetone at a concentration of 50 per cent (v/v) and alcohol at 75 per cent (v/v) concentration precipitated most of the enzyme. $(NH_4)_2SO_4$ precipitated the enzyme at 60 per cent (w/v) concentration. Kaolin and alumina C γ adsorbed the enzyme below pH 7.5, and an active eluate could be obtained with M/15 $(NH_4)_2HPO_4$ (but not M/15 Na_2HPO_4).

All preparations showed α-amylase activity. However, this enzyme is not a component of the mucolytic enzyme system, as a purified preparation[14] showed no activity against either heat-sterilized saliva or citric acid-precipitated mucoid.

The ammonium phosphate eluate from alumina C γ was chosen as the most suitable preparation and has been used for investigating the properties and mode of action of salivary mucinase. This eluate usually had an activity of 8 to 16.

3. *Properties.*—

(a) Effect of pH: The enzyme is inactivated below pH 4.5 and above pH 11.0. The inactivation of mucinase by acid produced from fermentable carbohydrate present in chewing gum was shown. Of forty samples of chewing gum-stimulated saliva incubated overnight, only one showed mucolytic activity. In all cases, except this one, the pH was below 4.5

The enzymic activity decreased slightly with rise in pH from 6.0 to 8.0, the activity being measured by the decrease in precipitable carbohydrate on incubation.

(b) Temperature of inactivation: Salivary mucinase is inactivated after heating for two minutes at 55° C.

(c) Activators and inhibitors: The effect of several possible activators and inhibitors neutralized and in a final concentration of M/100 was tested by measuring the decrease in precipitable carbohydrate on incubation. The following results were obtained: *No effect:* toluene, thymol, chloroform, salicylate, hydroquinone, ascorbic acid, cysteine, fluoride, sodium iodosobenzoate, and phenylmercuric acetate. *Inhibitors:* cyanide, oxalate, thiocyanate, arsenate, pyrophosphate, Hg^{++}, iodoacetic acid, Calgon (sodium hexametaphosphate), Sequestrol M (disodium salt of ethylenediaminetetraäcetic acid). *Activators:* Mg^{++}.

Thiocyanate[15] and Mg^{++}[16] are present in saliva at a much lower concentration than was used in the tests; when these ions were tested at the biologic level they showed no effect.

B. Bacterial Mucolytic Enzymes

1. *Mucolytic Bacteria.*—Evidence for the presence of bacteria producing mucolytic enzymes has been obtained, and several organisms with mucolytic activity were isolated.

The following procedure was adopted for the isolation and testing of organisms:

The bacteria were isolated from saliva by plating it out on blood agar or MacConkey's medium (for gram-negative rods). Pure cultures were grown in nutrient broth and then inoculated into sterile viscous saliva. In later experiments sodium glycerophosphate was added to the saliva to assist growth. Rogers[17] found that glucose lowers the yield of hyaluronidase due to acid production, but that glycerophosphate (final concentration of 1 per cent) is a quite satisfactory enrichment.

The production of a mucolytic enzyme is indicated by a decrease in mucoid content (measured by a decrease in ACRA titer) on incubation. The mucolytic activity of the culture fluid against heated viscous saliva or isolated mucoid was also determined. Using this procedure, several organisms were isolated from samples of saliva. These organisms were: (1) a gram-positive

rod resembling *Bacillus subtilis,* S1; (2) a gram-positive coccus resembling Streptococcus, S2; (3) a gram-positive coccus resembling Staphylococcus, S3; (4) two gram-negative rods, R1, R2. All these organisms fermented starch, and all except S3 liquefied gelatin.

Other mucolytic organisms have been identified among the 750 pure cultures brought back to the Institute of Dental Research by the New Guinea Nutrition Survey party (1947). Of 150 strains of gram-negative rods, only 13 showed mucolytic activity. These organisms also were characterized by the ability to hydrolyze both starch and gelatin. Further tests were made on 80 strains of gram-positive rods and 50 strains of gram-positive cocci, but none of these showed mucolytic activity. The enzyme produced is exocellular, and it has been possible to obtain an active Seitz filtered preparation. However, Seitz filtering lowered the activity (8^+ to 4^+ in one case), and it appears that this enzyme is partly held back, as is hyaluronidase.[18]

All the mucolytic organisms were shown to be capable of utilizing glucosamine. Suspensions of twenty-four hour cultures were made in distilled water, and 0.5 ml. was added to 0.5 ml. M/100 glucosamine HCl and 0.5 ml. M/20 phosphate buffer (pH 7.5: the optimum pH for glucosamine utilization[19]). All the organisms decreased the glucosamine content in three hours.

2. *Variation in Mucolytic Activity.*—Broth cultures were found to be devoid of activity, and it was therefore necessary to use a medium of sterile saliva or broth to which saliva had been added. The mucolytic activity of each individual strain of organism was found to vary. An unsuccessful attempt was made to improve the activity by subculturing several times in sterile saliva or a mixture of 4 ml. of nutrient broth and 1 ml. saliva. The possibility that variation in pH affected the activity was investigated, but could not be substantiated.

DISCUSSION

It has been possible to obtain partially purified preparations of salivary mucinase, and the properties of the enzyme have been studied. The isolated enzyme has similar properties to the enzyme of whole saliva and the conclusions from in vitro experiments with the isolated enzyme may thus be applicable to conditions in the mouth. It has been shown that the pH of saliva can on incubation fall low enough to inactivate the mucinase. In cavities, where the pH can fall to 4.2 following ingestion of fermentable carbohydrate,[20] the mucinase may thus be inactivated. Experiments previously reported[7] suggested that at this pH also the salivary mucoid would be precipitated. Although too low a pH will inactivate salivary mucinase, it has been found that enzymic activity increases slightly as the pH falls from 8.0 to 6.0. However, the difference in activity is so slight that it is probably not important in vivo.

The study of possible activators and inhibitors has shown that magnesium activates the mucinase system, and inhibition by cyanide, oxalate, pyrophosphate, Calgon, and Sequestrol M (all of which will bind calcium or magnesium) supports this finding. However, magnesium at the biologic level had no ef-

fect, so it is unlikely that the enzymic activity in vivo is affected by this element. Inhibition by iodoacetic acid and the mercuric ion suggested the presence of a sulfhydryl enzyme system. This possibility was eliminated, however, by the fact that neither iodosobenzoate nor phenylmercuric acetate were inhibitors. Simmons[1] reported that the depolymerization of salivary mucoid (as measured by a decrease in the viscosity of saliva) was not affected by toluene, thymol, chloroform, or fluoride at low concentrations whereas the mercuric ion, and also iodine, inhibited. His results have been confirmed in the present work.

Several organisms present in saliva have been shown to produce an enzyme, or enzymes, capable of depolymerizing salivary mucoid, and it appears that the majority of strains of mucinase-producing bacteria are gram-negative rods. These organisms were all shown to contain both proteolytic and amylolytic enzymes, and a relationship may, therefore, exist between the ability to hydrolyze starch and gelatin and the ability to depolymerize salivary mucoid. Such a relationship has been found in the case of bacterial hyaluronidase.[21]

The bacterial enzyme is exocellular, a cell-free culture filtrate having been found capable of depolymerizing salivary mucoid liberating the components. Thus, carbohydrate, a potential source of acid, can be produced in vivo, but whether or not the amount produced is significant is not known.

SUMMARY

The mucinase activity of saliva has been measured by three methods, viz., decrease in ACRA titer, decrease in precipitable carbohydrate, and decrease in viscosity.

The variation in enzyme titer has been investigated; it is concluded that the titer varies during the day and from day to day in one person, and that there is some variation from person to person.

Salivary mucinase has been partly purified by precipitation with acetone, alcohol, and ammonium sulfate, and adsorption onto kaolin or alumina C γ followed by elution with M/15 $(NH_4)_2HPO_4$.

The properties of salivary mucinase have been studied. The enzyme is inactivated below pH 4.5 and above pH 11.0; also heating at 55° C. for two minutes destroys the enzyme. Studies of possible activators and inhibitors suggest that the enzyme does not require the presence of sulfhydryl groups, but is activated by magnesium.

Several microorganisms with mucolytic activity have been isolated from saliva, and the production of an exocellular mucolytic enzyme has been demonstrated.

The results suggest that there is a relationship between the hydrolysis of starch and gelatin on the one hand, and the ability to depolymerize salivary mucoid on the other hand.

The mucolytic activity of single strains of bacteria has been found to vary unaccountably, and attempts to increase the mucolytic activity were unsuccessful.

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REFERENCES

1. Simmons, N. S.: The Existence of a Parotid Salivary Mucinase? *J. D. Res.* **20**: 255, 1941.
2. Rogers, H. J.: Bacterial Hydrolysis and Utilisation of Mucin in Saliva, *Nature, London* **161**: 815, 1948.
3. Meyer, K.: Mucoids and Glycoproteins, in Advances in Protein, Chemistry, Vol. 2, New York, 1945, Academic Press, Inc., p. 249.
4. Gore, J. T.: Saliva and Enamel Decalcification. III. Autolysis, *J. D. Res.* **17**: 411, 1938.
5. Knox, K. W.: Observations on the Action of Mucolytic Enzymes on Salivary Mucoid, *J. D. Res.* **32**: 374, 1953.
6. Gottschalk, A., and Lind, P. E.: Ovomucin: A Substrate for the Enzyme of Influenza Virus. I. Ovomucin as an Inhibitor of Haemagglutination by Heated Lee Virus, *Brit. J. Exper. Path.* **30**: 85, 1949.
7. Knox, K. W., and Still, J. L.: Observations on the Salivary Mucoids, *J. D. Res.* **32**: 379, 1953.
8. Burnet, F. M.: The Mucinase of *V. cholerae, Aust. J. Exper. Biol. & M. Sc.* **26**: 71, 1948.
9. Glass, G. B. J., and Boyd, L. J.: The Relation of Mucoid of Visible Gastric Juice and Its Split Products and Salivary Mucin to Dissolved Gastric Mucoproteose and Mucoprotein of Gastric Juice, *Bull. N. Y. M. Coll.* **12**: 1, 1949 (See *Chem. Abstracts* **44**: 4524, 1949.)
10. Dische, Z.: Ueber Microbestimmung der Kohlenhydrate in tierische Organen und im Blute mit Hilfe characteristischer Farben-reactionen, *Mikrochemie* **1**: 33, 1929.
11. Kabat, E. A., and Mayer, M. M.: Experimental Immunochemistry, ed. 1, Springfield, Ill., 1948, Charles C Thomas, p. 315.
12. Dewar, M. R.: Laboratory Methods for Assessing Susceptibility to Dental Caries. Part I. Effect of Variation in Technique, *D. J. Australia* **21**: 509, 1949.
13. Dreizen, S., Mann, A. W., Cline, J. K., and Spies, J. D.: The Buffering Capacity of Saliva as a Measure of Dental Caries Activity, *J. D. Res.* **25**: 213, 1946.
14. Meyer, K. H., Fischer, E. H., Staub, A., and Bernfeld, P.: Sur les enzymes amylolytiques. XI. Proprietes de l'amaylase crystallisee de saliva humaine, *Helvet. Chim. Acta* **31**: 2165, 1948.
15. Fischman, E. S., and Fischman, A.: Thiocyanate Content of Saliva of Normal and Hypertensive Subjects, *J. Lab. & Clin. Med.* **33**: 772, 1948.
16. Krasnow, F., and Oblatt, E. O.: Salivary Magnesium, *J. D. Res.* **19**: 305, 1940.
17. Rogers, H. J.: Simplified Culture Media for Production and Purification of Hyaluronidase, *J. Path. & Bact.* **56**: 284, 1951.
18. Russel, B. E., and Sherwood, N. P.: Hyaluronidase in Experimental Streptococcal Infection, *J. Infect. Dis.* **84**: 81, 1949.
19. Rogers, H. J.: Metabolism of Amino Acids. Breakdown of N-acetylglucosamine by Strains of *Streptococcus haemolyticus* and other Streptococci, *Biochem. J.* **45**: 87, 1949.
20. Stephan, R. M.: The Hydrogen Ion Concentration of the Dental Plaque, *J. D. Res.* **17**: 251, 1938.
21. Schwarbacher, G., Cunliffe, A. C., Williams, R. E. O., and Harper, G. J.: Hyaluronidase Production by Staphylococci, *Brit. J. Exper. Path.* **26**: 124, 1945.

OBSERVATIONS ON THE ACTION OF MUCOLYTIC ENZYMES ON SALIVARY MUCOID

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IN PREVIOUS papers, studies on the salivary mucoids[1] and mucolytic enzymes[2] have been reported, and their possible role in the etiology of dental caries has been discussed. In this paper the action of the salivary mucolytic enzyme system on its substrate is considered.

The salivary mucolytic enzyme system is probably composed of several enzymes, each of which is required to play its part before the salivary mucoid is finally depolymerized to its component sugars and amino acids. This investigation has been confined to a study of the carbohydrate moiety, and both the total carbohydrate and reducing sugar liberated have been estimated. In addition, as the enzyme preparation was not pure, the presence of other enzymes which may have mucolytic activity was determined, and these enzymes were then tested for their activity against salivary mucoid.

MATERIALS AND METHODS

Salivary mucoid[1] and the mucolytic enzyme, or mucinase,[2] were prepared as described previously. "Depolymerized mucoid" for testing the mucopolysaccharase activity of various enzymes was obtained by incubating the mucoid preparation with mucinase till the ACRA (Congo red in acid alcohol) titer[3] was zero. The enzyme was then heat-inactivated or removed by adsorption onto kaolin.

As elsewhere discussed,[2] a method has been developed for measuring the mucolytic activity, in which the decrease in carbohydrate precipitable by trichloracetic acid (T.C.A.) is determined. It was found that the presence of ammonium phosphate interfered with the formation of a firm clot on the addition of the precipitate. However, a dialyzed freeze-dried preparation of mucolytic enzyme dissolved in 0.9 per cent NaCl did not interfere with clotting.

Reducing sugar was estimated by the micromethod of Nelson[4] and the submicromethod of Park and Johnson.[5] It was not possible to obtain a precipitant suitable for the estimation of both total carbohydrate and reducing sugar. Heavy metal precipitants interfere with the total carbohydrate estimation and T.C.A. interferes with the reducing sugar methods. The precipitant recommended by Young[6] was used for removing mucoid and protein before

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estimating reducing sugar by the Nelson method. This precipitant was not suitable for the submicromethod, and the tungstate reagent of Horvarth and Knehr[7] has been used. Tungstate did not precipitate salivary mucoid, but was suitable when reducing sugar liberated from the depolymerized mucoid preparation was being determined; when estimating the reducing sugar, 2 ml. of precipitant were added to 1 ml. of preparation and, after centrifuging, the supernatant was neutralized with 3 drops of 0.5 per cent Na_2CO_3.

EXPERIMENTAL

1. *Action of Salivary Mucinase on Salivary Mucoid.*—The decrease in T.C.A.-precipitable carbohydrate and in reducing groups brought about by enzymic action was determined.

(a) Liberation of carbohydrate: A mixture of 3.5 ml. of salivary mucoid preparation (ACRA titer 4+) and 0.5 ml. of freeze-dried enzyme (10 mg./ml.—titer 16) was incubated for two hours. The amount of T.C.A.-precipitable carbohydrate (expressed as glucose) decreased from 32 μg per milliliter mucoid to 2 μg per milliliter mucoid, and the ACRA titer decreased from 4+ to zero. Thus, the results obtained with whole saliva have been confirmed.[2]

(b) Liberation of reducing groups: Whereas the liberation of carbohydrate could be shown in two hours, the liberation of reducing groups occurred more slowly and an incubation period of up to twenty hours (in the presence of toluene) was necessary. Three volumes of salivary mucoid preparation (ACRA titer 4+) and one volume of salivary mucinase ($[NH_4]_2HPO_4$ eluate—titer 8+) were mixed and incubated at 37° C., and the reducing sugar content of the protein-free filtrate was estimated by the method of Nelson.[4]

The results are expressed as μg glucose per milliliter mucoid (Table I).

TABLE I

LIBERATION OF REDUCING GROUPS BY ENZYMIC ACTION

TIME (HOURS)	ACRA TITER	REDUCING SUGAR (μG)	INCREASE IN REDUCING SUGAR (μG)
0	4	4	–
3	0	12	8
20	0	34	30

This and other similar experiments showed that reducing groups are liberated when the enzyme acts on the salivary mucoid preparation. Detectable amounts of reducing groups (8 μg glucose per milliliter mucoid) are liberated during the primary stage of enzymic action when the viscosity is being decreased. The further increase on longer incubation (22 μg glucose per milliliter mucoid) showed that the mucopolysaccharide moiety is being depolymerized, thus liberating the reducing sugar component.

2. *The Presence of Other Enzymes in the Mucinase Preparation.*—As all preparations of salivary mucinase showed α-amylase activity,[2] the possibility that still other enzymes were present was investigated. Hyaluronidase was shown to be present, but lysozyme and trypsin could not be detected. Lyso-

zyme would, however, have been inactivated by the akalinity of the eluant.[8]

3. *Action of Various Enzymes on Salivary and Depolymerized Mucoid.*—

α-Amylase,[9] hyaluronidase (Kinadin), lysozyme,[10] and trypsin* were tested for mucolytic activity upon the salivary mucoid preparation and depolymerized mucoid.

(a) Salivary mucoid: Trypsin was the only enzyme of those tested which decreased the viscosity of the mucoid preparation. This enzyme also depolymerized ovomucin.

(b) Depolymerized mucoid: Hyaluronidase was shown to liberate reducing sugar, but neither lysozyme nor α-amylase showed such activity.

As trypsin depolymerizes salivary mucoid, and hyaluronidase liberates reducing sugar from mucinase-depolymerized mucoid, the liberation of reducing sugar from trypsin-depolymerized mucoid was also investigated.

Incubation of the mucoid preparation with trypsin decreased the viscosity, but there was no liberation of reducing groups. Subsequent incubation with hyaluronidase did result in the liberation of reducing groups.

DISCUSSION

It has been shown that the decrease in viscosity of salivary mucoid (as exemplified by reduction in ACRA titer) on incubation with salivary mucinase is accompanied by the liberation of carbohydrate. As trypsin is capable of decreasing the viscosity of both the salivary mucoid preparation and ovomucin, and as trypsin is known to be an endopeptidase which acts at the carboxyl end of lysine and arginine residues,[11] the primary depolymerization apparently occurs in the peptide chain. Both lysine and arginine are present in saliva,[12] and arginine, because of its relatively high concentration in saliva and its presence in submaxillary mucoid,[13] may be an important component of the peptide moiety. The depolymerization of salivary mucoid by trypsin supports this suggestion.

As reducing sugar is detectable following the primary depolymerization, hydrolysis of some glycosidic bonds must have occurred. Hyaluronidase is not capable of decreasing the viscosity of salivary mucoid, although it will liberate reducing sugar from trypsin-depolymerized mucoid; this suggests that before hydrolysis of glycosidic bonds, a masking peptide must be removed. From the results of Meyer and his co-workers[14] on the mode of action of hyaluronidase, it may be concluded that this enzyme splits the glycosidic linkage between the amino sugar and the adjacent sugar, liberating the reducing group of the amino sugar.

Several enzymes have been investigated for mucolytic activity. It appears possible from the experiments described previously that hyaluronidase could play an important part in the degradation of salivary mucoid. However, Lisanti[15] has found hyaluronidase to be absent from the saliva of 25 per cent of people, suggesting that this enzyme is not an essential component of

*British Drug Houses Ltd.

the mucolytic enzyme system. It is possible that in these 25 per cent of people hyaluronidase is present but the activity very low, corresponding to the low mucinase titers which have been found.[2]

Although a proteolytic enzyme (trypsin) could not be detected, trypsin can decrease the viscosity of salivary mucoid. Apparently trypsin will partly hydrolyze the peptide moiety of the mucoid.

α-Amylase, which was present in the enzyme preparation, did not show mucolytic activity. Gore[16] has claimed that amylase is responsible for the decrease in viscosity of saliva, but his results could not be confirmed.

Simmons[17, 18] has obtained evidence which suggests that lysozyme can form a complex with salivary mucoid. The ressults obtained indicate that lysozyme has no action on salivary mucoid, so that in such a complex the mucoid would not be depolymerized.

Besides the enzymes which have been discussed, others are probably components of the mucolytic enzyme system. Peptidases would be required to liberate the components of the peptide moiety, and a specific enzyme may be required to split the linkage between the amino acid and carbohydrate residues.

SUMMARY

The mode of action of salivary mucinase has been studied and the liberation of carbohydrate and reducing sugar shown.

The enzyme preparation showed α-amylase and hyaluronidase activity; trypsin and lysozyme were not detected. Trypsin decreased the viscosity of salivary mucoid, and hyaluronidase liberated reducing sugar from mucinase- or trypsin-depolymerized mucoid.

It is suggested that the decrease in viscosity of salivary mucoid is due to the action of a proteolytic enzyme and that subsequently a hyaluronidase-like enzyme liberates the components of the mucopolysaccharide. Other enzymes are probably required before complete depolymerization occurs.

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REFERENCES

1. Knox, K. W.: Observations on the Salivary Mucoids, *J. D. Res.* **32**: 379, 1953.
2. Knox, K. W.: Observations on the Salivary Mucolytic Enzymes, *J. D. Res.* **32**: 367, 1953.
3. Burnet, F. M.: The Mucinase of *V. cholerae*, *Australian J. Exper. Biol. M. Sc.* **26**: 71, 1948.
4. Nelson, N.: Determination of Glucose. Photometric Adaptation of Somogyi Method, *J. Biol. Chem.* **153**: 375, 1944.
5. Park, J. J., and Johnson, M. J.: Submicro-determination of Glucose, *J. Biol. Chem.* **181**: 149, 1949.
6. Young, D.: Salivary Sugar Analysis, Past and Present Methods and the Results of Sugar Analysis of Saliva, *J. D. Res.* **20**: 597, 1941.
7. Horvarth, S. M., and Knehr, C. A.: Adaptation of Folin Malmros Micro Blood Sugar Estimation, *J. Biol. Chem.* **140**: 869, 1941.
8. Hawthorne, J. R.: Action of Egg White Lysozyme on Ovomucin and Ovomucoid, *Biochim. et biophys. acta* **6**: 28, 1950.

9. Meyer, K. H., Fischer, E. H., Staub, A., and Bernfeld, P.: Sur les enzymes amylolytiques. X. Isoelement et crystallisation de l'α-amylase de saliva humaine, *Helvet. Chim. Acta* **31**: 2158, 1948.
10. Carter, H. E. (Ed.): Biochemical Preparations, Vol. 1, New York, 1949, John Wiley & Sons, Inc., p. 67.
11. Bergman, M., and Fruton, J. S.: The Specificity of Proteinases, in Advances in Enzymology, Vol. 1, New York, 1941, Interscience Publishers, Inc., p. 63.
12. Kesel, R. G., O'Donnell, J. F., Kirch, E. R., and Wach, F. C.: Amino Acids in Human Saliva, *J. D. Res.* **26**: 297, 1947.
13. Meyer, K.: Mucoids and Glycoproteins, in Advances in Protein Chemistry, Vol. 2, New New York, 1945, Academic Press, Inc., p. 249.
14. Rapport, M. M., Meyer, K., and Linker, A.: Correlation of Reductimetric and Turbidimetric Methods of Hyaluronidase Estimation, *J. Biol. Chem.* **186**: 615, 1950.
15. Lisanti, V. F.: Hyaluronidase Activity in Human Saliva, *J. D. Res.* **29**: 392, 1950.
16. Gore, J. T.: Saliva and Enamel Decalcification. III. Autolysis, *J. D. Res.* **17**: 411, 1938.
17. Simmons, N. S.: Studies on Salivary Mucopolysaccharides. Lysozyme Polysaccharide Complex, *J. D. Res.* **28**: 640, 1949.
18. Simmons, N. S.: Studies on the Defence Mechanisms of the Mouth, *J. D. Res.* **30**: 494, 1951.

OBSERVATIONS ON THE SALIVARY MUCOIDS

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IN ANY consideration of dental caries the saliva needs to be taken into account, and the present investigation concerns one of its principal components, the mucoid. This component is mainly responsible for the viscosity of saliva, and it is of further interest as it is a possible source of nutrients for the oral flora, containing both carbohydrate and amino acids.

Following Meyer's classification[1] the term "mucoid" will be used, denoting a protein-polysaccharide complex, in preference to other terms which have been used synonymously in the past, viz., mucin, mucoprotein, and glycoprotein. The literature on the salivary mucoids has been reviewed recently[2, 3] and, accordingly, the salivary mucoids will be considered here only briefly.

The mucoids of saliva are secreted by the sublingual, submaxillary, and small glands of the oral mucosa,[4] and the mucoid content has been estimated by different methods to be between 0.05 and 0.60 per cent.[5-7] Various workers[8-11] have investigated the possibility of a relationship between caries susceptibility and the mucoid content or viscosity, but no correlation could be found. However, this may be due to variation in one person or to faulty technics. In any method for estimating the mucoid content, the presence of an enzyme system causing a rapid depolymerization of the mucoid[12-14] must be taken into account.

Although the salivary mucoids have been little studied, there is evidence that there are at least two components. Glass and Boyd[15] found that 8.8 per cent of the total mucoid in human saliva is not precipitated by trichloroacetic acid, and in cats' submaxillary saliva two distinct mucoids have been shown.[16] Meyer (in a private communication to Clement[2]) "stresses the mixed nature of the mucoprotein fraction of saliva and points out that it is not yet known whether any of the subsidiary fractions contain glucuronic acid, but it is certainly absent from the main fraction. The latter, however, does contain gluconic acid."

The submaxillary gland contains two mucoids,[17] viz., an acidic one, composed of N-acetylglucosamine and gluconic acid, and a neutral one containing glucosamine and mannose (ratio 1:2). The mucoid of the sublingual gland also contains glucosamine and mannose in the ratio of 1:2.[18] It is tempting to suggest that the main mucoid fraction of saliva is the same as the acidic mucoid of the submaxillary gland; however, Clement[2] pointed out that

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the components of the secretory granules differ from the final product in the secreted fluid, and are to be regarded as the precursors of the substance in saliva.

In this investigation the variation in mucoid content which can occur during the day in one person was first studied. Some of the properties of the mucoids in whole saliva were then investigated and compared with those of a preparation obtained from saliva. Attempts were made to prepare the polysaccharide moiety by tryptic digestion, but these, however, proved unsuccessful. Apparently, as claimed by Asagi,[19] the peptide moiety is only partially attacked by trypsin.

METHODS

In order to show a relationship (should such exist) between caries susceptibility and salivary mucoid content, a rapid and accurate method of determining mucoid is required. Burnet[20] developed a quick method (ACRA [Congo red in acid alcohol] method) depending on the precipitability of mucoids in an acid-alcohol mixture. This method was chosen as the most suitable because the breakdown of mucoid by depolymerizing enzymes on standing is avoided and, unlike other methods, it measures only fully polymerized mucoid. Dewar[21] compared the ACRA titer of saliva with the mucoid value obtained by chemical methods, but found no correlation. Several mucoids are capable of inhibiting hemagglutination,[22] the inhibitor being called a Francis inhibitor.[23] The ability of whole saliva to inhibit hemagglutination was shown by Francis and Minuse,[24] and it has been further studied by the method developed by Burnet.[25]

EXPERIMENTAL

1. Variation in the Mucoid Content of Saliva.—

The ACRA titer: The ACRA titer of freshly collected samples of resting saliva from one individual (K. W. K.) was determined over two successive days, the titer being estimated hourly between 7 A.M. and 8 P.M.

The results showed no constancy, although no food or drink was taken between meals and the corresponding meals each day were substantially the same in nature. The titer varied between 4 and 128+, being 64 or greater in 50 per cent of cases, and the titers at any particular time of day agreed only twice.

The Francis inhibitor titer: The Francis inhibitor (F.I.) titer of saliva was also shown to vary during the day, although the variation in titer (400 to 800) was not as great as the variation in ACRA titer. The ratio of ACRA titer to F.I. titer was not constant, so that the F.I. titer is not a measure of the total mucoid content.

2. Preparation of Salivary Mucoid.—Precipitation of the mucoids of saliva with ammonium sulfate, weak acids, acetone, and alcohol was studied in an attempt to obtain the whole of the mucoid in a fully polymerized state, and in a suitable form for the study of mucolytic enzyme action. As the

mucoids are being continually broken down, the saliva should be ejected directly into a tube containing the precipitating fluid. Precipitation with weak acids (final concentration approximately M/50) proved advantageous, as most of the mucoid clot floated and could be removed easily following centrifuging; a small amount of mucoid was thrown down with the salivary debris, but no tests have been carried out on this material. Of the weak acids used, citric acid was chosen as most suitable for further work, as it gave the highest yield of floating precipitate. The preparation, after washing well with 0.9 per cent NaCl, slowly dissolved in 0.5 per cent Na_2CO_3 to give a viscous solution which was then neutralized with HCl. This preparation was readily depolymerized by salivary mucinase.

3. Properties.—In this section some of the properties of the mucoids in whole saliva are compared with those of the product obtained by citric acid precipitation. The term "salivary mucoid" will be used in referring to the mucoids in whole saliva and the term "mucoid preparation" in referring to the isolated product.

(a) Effect of heat: Salivary mucoid was not affected by heating for thirty minutes at temperatures up to 90° C., but the ACRA titer fell from 8 to 0 after fifteen minutes at 100° C. The ACRA titer of the mucoid preparation decreased to 0 after thirty minutes at 70 to 75° C., and more quickly at higher temperatures.

(b) Precipitation: The mucoid preparation, like salivary mucoid, is precipitated by weak acids at pH 4.5 to 5.0 but, whereas $N_{/10}$ HCl will precipitate the mucoid preparation, it will not precipitate salivary mucoid. Calcium ions are bound to salivary mucoid,[26] and so precipitation by weak acids may be due to removal of calcium ions. Confirmatory evidence for this suggestion could not be obtained, however, as attempts to remove the calcium and find whether calcium-free preparation resisted precipitation by weak acids proved unsuccessful. Ethylenediaminetetraäcetic acid, which is a very efficient calcium binder,[27] was precipitated by acidification before salivary mucoid.

(c) Buffering capacity: The buffering capacity of the mucoid preparation has been compared with that of dialyzed and enzyme depolymerized mucoid. Dewar[28] found that only in people not susceptible to caries is more than 0.76 ml. $N_{/50}$ lactic acid required to lower the pH of 1 ml. saliva from 7.0 to 6.0 On the other hand, 1 ml. of the mucoid preparation (obtained from persons of varying dental status) required 0.72 ml. This appeared to indicate that mucoid accounted for most of the buffer capacity of saliva. However, dialysis lowered the buffering capacity considerably, and so the good buffering capacity may have been due to adsorbed salts. The addition of 0.5 per cent NaCl to the dialyzed material did not increase the buffering capacity. Tests in hog gastric mucin, on the other hand, showed that the addition of NaCl to a dialyzed solution did increase the buffering capacity. The mucoid preparation was incubated at 37° C. with salivary mucinase (a freeze-dried preparation in water, obtained as described elsewhere[14]), and the buffering capacity was determined at once and after three hours. There was a 10 per cent increase following incubation.

4. Analysis of Salivary Mucoid.—The mucoid preparation contained 7.0 per cent total nitrogen[29] and 34 per cent hexosamine.[30] The hexosamine content accounts for 2.6 per cent of the total nitrogen, so that there is 4.4 per cent protein nitrogen present.

The absence of uronic acid[2] was confirmed by the naphthoresorcinol test.[31]

Phosphorus (0.3 per cent) was shown to be present by the method of Fiske and Subbarow[32] and 0.5 per cent sulfur was determined, using an acid hydrolysate[17] of the mucoid preparation.

DISCUSSION

The ACRA method[20] of determining the mucoid content of saliva was used, rather than measurement of viscosity, because the relative viscosity has been shown to decrease rapidly on standing, and other methods were not considered satisfactory. The results obtained indicate that there is a wide variation throughout the day in the mucoid content of saliva as measured by the ACRA method, and Dewar[21] has found that "even when collected under standard conditions" there is still a wide variation. Unless the variations encountered are mainly due to faults in technic which can, eventually, be eliminated by standardizing the methods of sampling and estimation of mucoid content, it will not be possible to determine whether there is any relationship between caries susceptibility and the mucoid content of saliva. The mode of formation of dental plaque and its attachment to the enamel surface are little understood. Ennever, Robinson, and Kitchin[33] concluded from their studies that it is very probable that Actinomyces species form the structural basis for the plaque, though "the possibility that the altered mucin could initiate plaque formation remains open." Mucoid may be an important component of the plaque, and two methods of denaturation have been suggested, viz., alternate wetting and drying,[34] and acid precipitation.[35]

Although acid precipitation in vitro gives a flocculent precipitate, this method of precipitation under certain conditions may be the mechanism of deposition of the plaque, and the fact that there is an increased rate of plaque formation during the ingestion of food,[36] when acid is produced by fermentation, is supporting evidence. According to Inouye[5] the isoelectric point of salivary mucoid is pH 2.75 to 2.95, but it has been shown that it is not necessary to reach this value for precipitation to occur. Weak acids, such as citric acid, tannic acid, and lactic acid, all of which would be present in the saliva at times, cause precipitation of salivary mucoid at a pH between 4.5 and 5.0. These values are possible in the mouth. Stephan[37] studied the pH of plaques and cavities, and found that of the plaques to vary between 4.6 and 7.0, and that of the cavities between 4.2 and 6.2.

Studies on the buffering capacity of isolated mucoid suggest that in vivo the buffering capacity of mucoid may be important. Thus, a higher mucoid content may lead to a higher buffering capacity. However, the results obtained cannot be applied to saliva without reservation, because the isolated mucoid was in a solution of higher salt (NaCl) concentration than saliva and

may, in addition, have contained adsorbed salivary salts. Dialysis was found to cause a marked decrease in buffering capacity.

In saliva, Sellman[38] concluded that bicarbonate and phosphate are the principal buffers at physiologic levels, but that at lower hydrogen-ion concentrations they are replaced by the proteins. In the plaque the buffering capacity is considered substantially due to protein,[39] part of which would be mucoid from saliva. Fosdick[36] stated that the proteins in saliva would have a tendency to buffer acids, and points out that from the chemical point of view it would be difficult to differentiate between protein or bacteria and that of precipitated mucin or other protein from saliva. Thus, it seems probable that mucoid plays a part in the buffering capacity of saliva and of the plaque.

The increase in the buffering capacity of saliva on standing has been noted by Dewar,[40] and it appears that this increase is partly due to the increase in buffering capacity of salivary mucoid on depolymerization. Thus, the activity of the mucolytic enzyme system may affect the buffering capacity of saliva.

It is probable that the product isolated by acid precipitation is, like that of Glass and Boyd,[15] the principal mucoid of saliva. Some differences have been noted between this preparation and salivary mucoid which may be due to the fact that, whereas the product in vivo is believed to be a calcium mucinate complex, the isolated product would be mainly a sodium mucinate. However, the mucoid preparation gives a viscous solution which is readily depolymerized by salivary mucinase, and this product proved a satisfactory substrate for subsequent studies on the salivary mucolytic enzyme system.

SUMMARY

The ACRA titer of saliva has been determined as a measure of mucoid content. It has been shown that there is a wide variation in the mucoid content of saliva from one person.

Preparations of mucoid which redissolve to give viscous solutions, and thus have not been depolymerized to any extent, have been obtained. The precipitate obtained with citric acid has been chosen as the most suitable.

Some of the properties of the mucoid of whole saliva and the mucoid preparation have been investigated; the isolated product differs from the salivary mucoid in stability to heat and in precipitability by weak acids.

The buffering capacity of the mucoid preparation was decreased by dialysis and increased on enzyme depolymerization. Analysis of the mucoid preparation showed 7.0 per cent total nitrogen and 34 per cent hexosamine.

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REFERENCES

1. Meyer, K.: Mucoids and Glycoproteins, in Advances in Protein, Chemistry, Vol. 2. New York, 1945, Academic Press, Inc., p. 249.

2. Clement, A. J.: The Chemical Composition of Salivary Mucin (Mucoprotein), *J. D. A. South Africa* **6**: 277, 1951.
3. Knox, K. W.: The Mucins and Mucoids in Relation to Dental Problems, *Austral. J. Den.* **55**: 351, 1951.
4. Babkin, B. P.: The Physiology of the Salivary Glands, in Dental Science and Dental Art, Philadelphia, 1938, Lea & Febiger, p. 219.
5. Inouye, J. M.: Salivary Mucin, *J. D. Res.* **10**: 76, 1930.
6. Glass, J.: Eine Quantitative chemische Methode zur Bestimmung des Mucin—(Schleim)—Gehaltes in Magenshaft, Speichel und Sputum, *Mikrochemie* **26**: 95, 1939.
7. Dobbs, E. C.: Local Factors in Dental Caries, *J. D. Res.* **12**: 853, 1932.
8. Lothrop, A. P., and Gies, W. J.: A Chemical Study of Saliva and Its Probable Relation to Decay of the Teeth, *J. Allied Dent. Soc.* **6**: 65, 1911.
9. Karshan, M.: Factors in Human Saliva Correlated With the Presence and Activity of Dental Caries, *J. D. Res.* **15**: 383, 1936.
10. Rae, J. J., and Clegg, C. T.: The Relation Between Buffering Capacity, Viscosity and Lactobacillus Count of Saliva, *J. D. Res.* **28**: 589, 1949.
11. Willsmore, N. M.: Study of the Osmotic Pressure and Viscosity of Saliva and the Relation to Common Oral Conditions, *Austral. J. Den.* **41**: 161, 1937.
12. Simmons, N. S.: The Existence of a Parotid Salivary Mucinase? *J. D. Res.* **20**: 255, 1941.
13. Rogers, H. J.: Bacterial Hydrolysis and Utilization of Mucin in Saliva, *Nature, London* **161**: 815, 1948.
14. Knox, K. W.: Observations on the Salivary Mucolytic Enzymes, *J. D. Res.* **32**: 367, 1953.
15. Glass, G. B. J., and Boyd, L. J.: The Relation of Mucoid of Visible Gastric Juice and Its Split Products and Salivary Mucin to Dissolved Gastric Mucoproteose and Mucoprotein of Gastric Juice, *Bull. New York M. Col.* **12**: 1, 1949; see *Chem. Abstracts* **44**: 4524, 1949.
16. Komarov, S. A., and Stavraky, G. W.: The Nitrogenous Constituents of Cat's Saliva Evoked by Parasympathetic and Sympathetic Stimulation, *Canad. J. Research*, Sect. D. **18**: 233, 1940.
17. Blix, G.: Ueber die Kohlenhydratgruppen des Submaxillarismucins, *Ztschr. f. physiol. Chem.* **240**: 43, 1936.
18. Tanabe, Y.: Biochemical Studies on Carbohydrate, XLIX. Submaxillary Mucin and Mucoid, *J. Biochem.* **30**: 181, 1939.
19. Asagi, Y.: Nature of the Protein in Natural Glycoproteins, *Tohoku J. Exper. Med.* **50**: 95, 1949; see *Chem. Abstracts* **44**: 4055, 1950.
20. Burnet, F. M.: The Mucinase of *V. cholerae*, *Australian J. Exper. Biol. & M. Sc.* **26**: 71, 1948.
21. Dewar, M. R.: Personal Communication.
22. Anderson, S. G., Burnet, F. M., Fazekas de St. Groth, S., McCrea, J. F., and Stone, J. D.: Mucins and Mucoids in Relation to Virus Action. V. General Discussion, *Australian J. Exper. Biol. & M. Sc.* **26**: 403, 1948.
23. Francis, T., Jr.: Dissociation of Haemagglutinating and Antibody Measuring Capacities of Influenza Virus, *J. Exper. Med.* **85**: 1, 1947.
24. Francis, T., Jr., and Minuse, E.: Influence of Saliva on Haemagglutination of Influenza Virus, *Proc. Soc. Exper. Biol. & Med.* **69**: 291, 1948.
25. Burnet, F. M.: Mucins and Mucoids in Relation to Influenza Virus Action. III. Inhibition of Virus Haemagglutination by Glandular Mucins, *Australian J. Exper. Biol. & M. Sc.* **26**: 371, 1948.
26. Krasnow, F., Oblatt, E. O., and Rechnitzer, V.: Dialysis and Adsorption Studies on Salivary Calcium, Phosphorus and Magnesium, *J. D. Res.* **15**: 217, 1935.
27. Schwarzenbach, G., and Ackerman, H.: The Homologues of Ethylenediamine-tetraacetate and Its Alkaline Earth Complexes, *Helvet. Chim. Acta* **31**: 1029, 1948.
28. Dewar, M. R.: Laboratory Methods for Assessing Susceptibility to Dental Caries, Part I. Effect of Variation in Technique, *D. J. Australia* **21**: 509, 1949.
29. Ma, T. S., and Zuazaga, G.: Micro-Kjeldahl Determination of Nitrogen, *Indust. & Engin. Chem. (Anal. Ed.)* **14**: 280, 1942.
30. Elson, L. A., and Morgan, W. T. J.: Colorimetric Determination of Glucosamine and Chondrosamine, *Biochem. J.* **28**: 988, 1934.
31. Hawk, P. B., Oser, B. L., and Summerson, W. H.: Practical Physiological Chemistry, ed. 12, Philadelphia, 1947, The Blakiston Company, p. 716.
32. Fiske, C. H., and Subbarow, Y.: The Colorimetric Determination of Phosphorus, *J. Biol. Chem.* **66**: 375, 1925.
33. Ennever, J., Robinson, H. B. G., and Kitchin, P. C.: Actinomyces and the Dentobacterial Plaque, *J. D. Res.* **30**: 88, 1951.
34. Dobbs, E. C.: Local Factors in Dental Caries, *J. D. Res.* **12**: 583, 1932.

35. Keiser-Nielson, H.: Role of Mucin in Pathogenesis of Caries, *Acta odont. Scandinav.* **7**: 97, 1946.
36. Fosdick, L. S.: Theoretical Considerations of Certain Phases of the Caries Problem, *Northwest. Univ. Bull.* **49**: 4, 1949.
37. Stephan, R. M.: The Hydrogen Ion Concentration of the Dental Plaque, *J. D. Res.* **17**: 251, 1938.
38. Sellman, S.: Buffer Value, of Saliva and Its Relation to Dental Caries, *Acta odont. Scandinav.* **8**: 244, 1949.
39. Stralfors, A.: Studies on the Microbiology of Caries, III. The Buffering Capacity of the Dental Plaques, *J. D. Res.* **27**: 587, 1948.
40. Dewar, M. R.: Laboratory Methods for Assessing Susceptibility to Dental Caries. Part II. Correlation of Results Obtained by Clinical Examination and by Standardised Laboratory Methods, *D. J. Australia.* **22**: 24, 1950.

HISTOLOGIC FINDINGS IN THE KIDNEY, LIVER, PANCREAS, ADRENAL, AND THYROID GLANDS OF THE RAT FOLLOWING SODIUM FLUORIDE ADMINISTRATION

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FLUORIDE intake, from the standpoint of its dental effects, has already been intensively studied on both a clinical and an epidemiological basis.[1-4] Information as to the sites of action and nondental effects of fluorides is incomplete. There has been little reported study of mammalian viscera after acute fluoride intoxication. Roholm[5] described "islands" of incipient necrosis in the liver, as well as renal hemorrhage, inflammation, and tubular degeneration after acute toxicosis. Histologic investigations after chronic fluoride administration, however, have been more comprehensive. From the clinical standpoint such studies are more pertinent, since they more nearly approach the conditions of fluoride assimilation in human beings.

The most extensive studies deal with alterations in the thyroid, adrenal, liver, kidney, and pancreas. Generally, the thyroid is reported as undergoing hypertrophy, hyperplasia, and increased colloid withdrawal.[6-13] Roholm[5] and Kick,[14] however, failed to confirm these findings. Although Sollmann, Schettler, and Wetzel,[15] Biester, Greenwood, and Nelson,[13] and Roholm[5] detected no alteration in adrenal glands, Phillips and Lamb[11] noted hyperemia and degenerative changes. The liver showed varying degrees of degeneration in the studies of Phillips and his co-workers,[16] Muehlberger,[17] and Velu and Zottner,[18] although again Roholm[5] and Kick and his associates[14] failed to confirm this.

Marked structural changes have been reported in the kidneys of animals acutely and chronically poisoned. In addition to hemorrhage, glomerular hyperemia, and widespread inflammatory change, three pathologic conditions have been noted consistently. These conditions are subacute glomerulonephritis (chronic parenchymatous nephritis), chronic nephritis (chronic interstitial nephritis), and nephrosis.[5, 14, 19]

Significant changes in other viscera have not been reported.

MATERIAL AND PROCEDURE

This experiment involved the same groups of animals and the same injection procedures as reported in an earlier paper.[27] Thus, two groups of rats of the Long-Evans strain were studied, each group consisting of ten experimental and five control animals. The two groups were designated as acute and

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chronic, based on the duration of the experimental period and amount of sodium fluoride administered. Rats of the acute group were 49 days old at the onset of injections, and those of the chronic group were 52 days old. All rats received a modified McCollum's diet. Animals of the acute group were weighed at two-day intervals, and those of the chronic group at eight-day intervals, throughout the experiment.

The intraperitoneal route of injection was selected because it permits an efficient absorption of salts from the peritoneum and also produces less local tissue destruction than results from injection elsewhere. Feeding of the agent would not have permitted accurate determination of the dosage.

A preliminary experiment was conducted to determine tolerance of sodium fluoride, in order to establish dosage levels. The dosage employed by Schour and Smith[20] in adult rats (0.3 c.c. of 2.5 per cent aqueous sodium fluoride) was lethal in these younger animals. Nonetheless, when virtually the same amount of the salt (6.9 mg. vs. Schour's 7.5 mg.) was administered in isotonic solution it was tolerated. The initial dose for the acute group was, therefore, at this level. Subsequent daily injections of isotonic sodium fluoride solution varied between 0.8 and 1.2 c.c. in order to maintain the rats in a state of acute toxicosis. The experimental period was fifteen days. During this time a total of 16.2 c.c. was injected, representing 406.47 mg. of the salt. The chronic group received injections of the isotonic solution on alternate days and in lower volumes over a 100-day period.

The dosage was altered according to the tolerance of the animals. For the first thirty days each rat received 0.5 c.c. every other day. The dosage was increased to 1.0 c.c., for the next eighteen days, then reduced for ten days to 0.6 to 0.8 c.c. Thereafter, it fluctuated between 0.9 c.c. and 1.6 c.c. The total received was 45.1 c.c. in 100 days, representing 1131.65 mg. of the salt.

Control rats in each group received injections of physiologically isotonic sodium chloride solution in volumes equal to those given the experimental rats.

At autopsy the viscera selected for study (liver, pancreas, adrenal, kidney, thyroid, and salivary glands*) were dissected free. Portions of all tissues were fixed in Bouin's solution. In addition, slices of liver were fixed in Carnoy II, portions of pancreas in Zenker-formol, and one of the adrenals in 10 per cent neutral formol. Adrenals and thyroids were weighed after fixation.

All tissues were embedded and sectioned in nitrocellulose at 7 micra thickness, except for frozen sections of the formalin-fixed adrenals. Iron hematoxylin aniline blue (I.H.A.B.)[21] was the general stain used, with Best's carmine[22] being applied to the liver for glycogen stains, and sudan III[23] to the adrenal for lipid distribution.

RESULTS

Seven fluoride-injected animals died during the experimental periods. One other animal survived the experimental period, but exhibited such bizarre overall changes that it could not be correlated. This animal was, therefore, omitted from further consideration. It is realized that the total number of animals in-

*The findings in the salivary glands have been reported separately.[17]

volved in the study was small indeed. Nonetheless, definite trends were observed in the experimental animals and these deserve mention.

Although weight gain was retarded in the treated animals, the differences which developed did not attain statistical significance (Table I).* Approximately one-half of the fluoride-injected animals of both groups showed skin lesions at the injection site, resulting from contact of sodium fluoride with superficial tissues. The classic gross effects of fluorine upon incisor enamel substance were noted, but not in constant degree.

TABLE I
BODY WEIGHTS OF RATS INJECTED WITH SODIUM FLUORIDE

EXPERIMENT	GROUP	BODY WEIGHT IN GRAMS*	
		AT ONSET	AT AUTOPSY
Acute	Control	163.5 ± 18.41	234.8 ± 18.38
	Fluoride injected	159.7 ± 3.7	203.0 ± 13.08
Chronic	Control	160.0 ± 16.08	395.4 ± 13.03
	Fluoride injected	178.4 ± 7.16	385.8 ± 7.61

*Mean and standard error of body weights.

Grossly, no abnormalities were evident in the viscera at autopsy. The remainder of this description, therefore, deals with histologic findings.

Kidney.—The most striking change, though seen only in the chronically injected group, was edema of the connective tissue between the tubules in the lower half of the papilla (Figs. 1 and 2). The connective tissue was vacuolated, rather than showing the aniline-staining collagenous fibers. The vacuoles themselves either were empty or contained a very pale blue-staining amorphous substance (Fig. 2). The tubules no longer maintained the parallel alignment characterizing this region in the normal kidney, and were apparently reduced in number. The walls of the tubule cells (chronic group) were distorted and the cell membranes poorly defined. Lastly, the cells of the straight collecting tubules were shrunken and less cuboidal, while their nuclear chromatin appeared more granular.

There was a greatly increased prominence of the blood vessels in the glomeruli of treated animals (six of the seven in the acute group, and four of the five in the chronic group). The glomerular capillaries were not only engorged with erythrocytes, but were also apparently increased in number (Figs. 3 and 4).

The vascularity of the medulla of the kidney was markedly increased in all but one of the treated animals (Figs. 5 and 6, compared with normal in Fig. 1). Capillaries were larger, more numerous, and engorged with erythrocytes.

*In the statistical analysis of data, the means and their standard errors were computed. All standard errors were corrected for small size of sample by the factor $\sqrt{\frac{n}{n-1}}$. Where a statistically significant difference between means is claimed, the ratio of the difference between the means to the standard error of that difference was 3 or greater (i.e., the index of significance of difference was 3 or greater). Standard statistical methods were followed as defined by Pearl.[34]

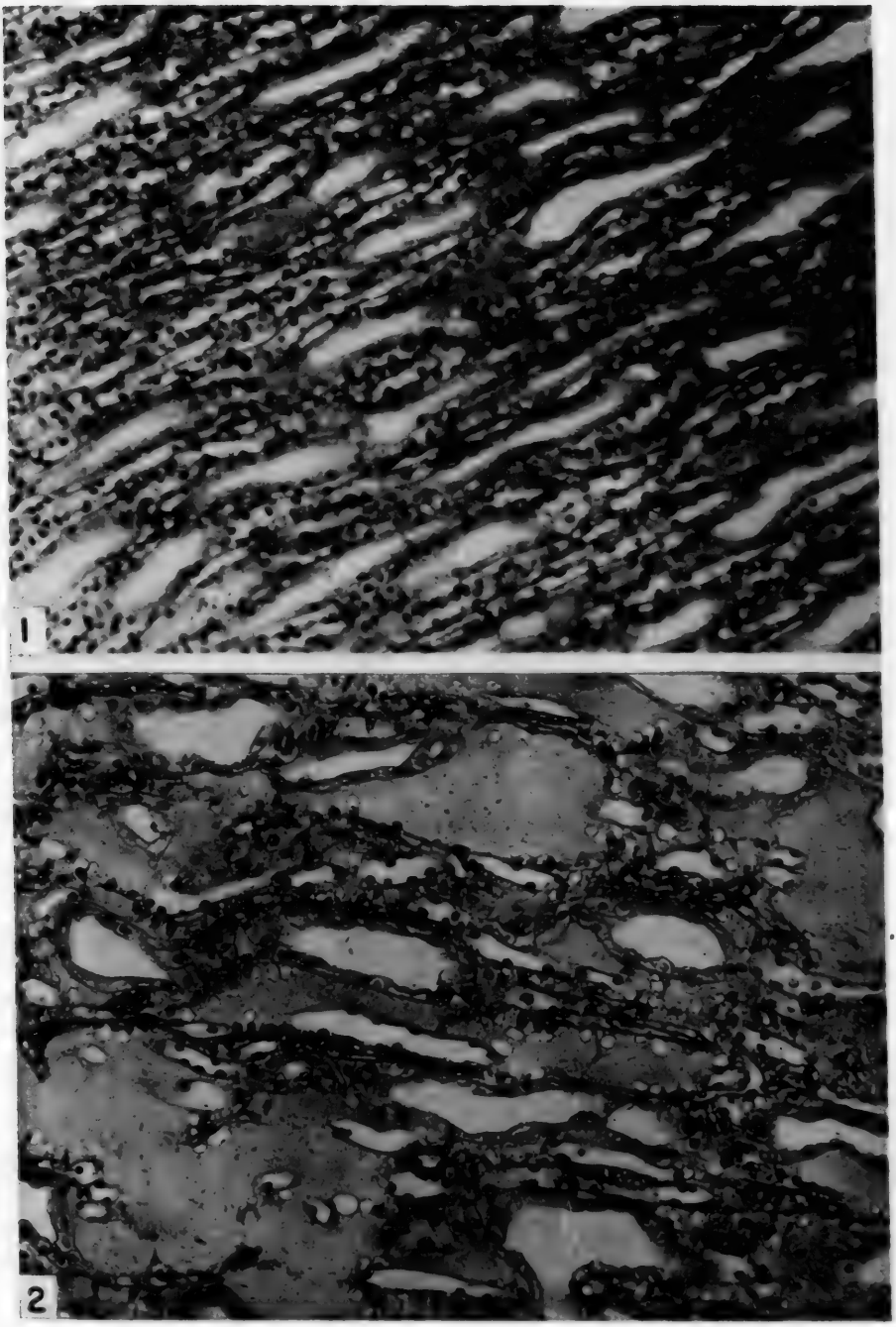


Fig. 1.—Kidney, medulla, and lower half of papilla. Control animal, chronic experiment. I.H.A.B. stain, ×190. Note (1) the regular and compact arrangement of the tubules, (2) the narrow bands of connective tissue between the tubules, and (3) the distinct outline of the tubule cells.

Fig. 2.—Kidney, medulla, and lower half of papilla. Sodium fluoride-injected animal, chronic experiment. I.H.A.B. stain, ×190. Note, as compared to the control (Fig. 1), (1) disruption of the normal parallel alignment of tubules, (2) the wide vacuolated areas which separate the tubules, (3) tubule remnants within vacuolated areas, (4) the apparent decrease in the number of tubules, and (5) disappearance of normal tubule cell outline.

No increased cell proliferation was found in the kidney.

Pancreas.—In the experimental animals the pancreatic interlobular and intralobular connective tissue septa were increased in width (Figs. 7 and 8). Although there was variability within the groups, mitotic activity could be seen

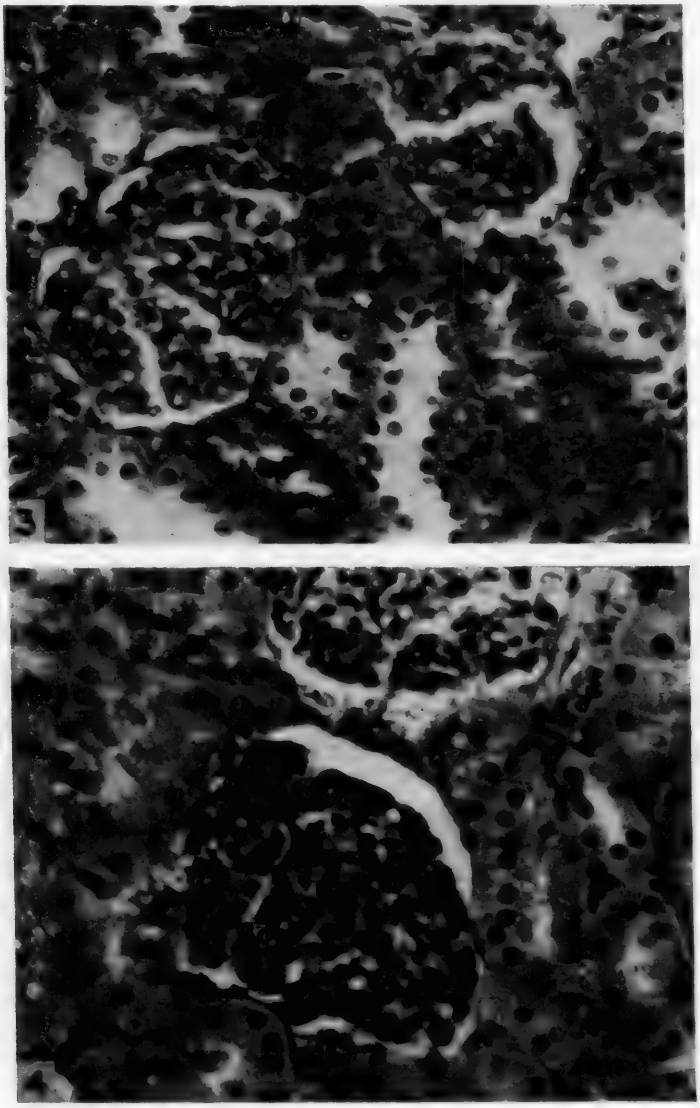


Fig. 3.—Kidney, cortex. Control animal, acute experiment. I.H.A.B. stain, ×400.

Fig. 4.—Kidney, cortex. Sodium fluoride-injected animal, acute experiment. I.H.A.B. stain, ×400. Note the increased prominence of the blood vessels in the glomeruli. The glomerular capillaries are not only engorged with erythrocytes, but are also apparently increased in number.

to be definitely increased in the majority of the experimental animals (Fig. 8 and Table II). No changes attributable to the fluorosis could be detected in nuclear size, or in the cytoplasm of alveolar cells. No significant proliferation could be seen in the islet cells.

Adrenal Gland.—The average weight of the adrenal glands (both right and left) of treated animals was greater than in the respective controls. The difference was probably significant in the rats on the acute regimen, where the index of significance was 2.17 for the left adrenals and 2.92 for the right adrenals (Table III). In this same acute group, the adrenal cortex was enlarged, although on planimetric measurement of histologic sections the increase was not statistically significant.

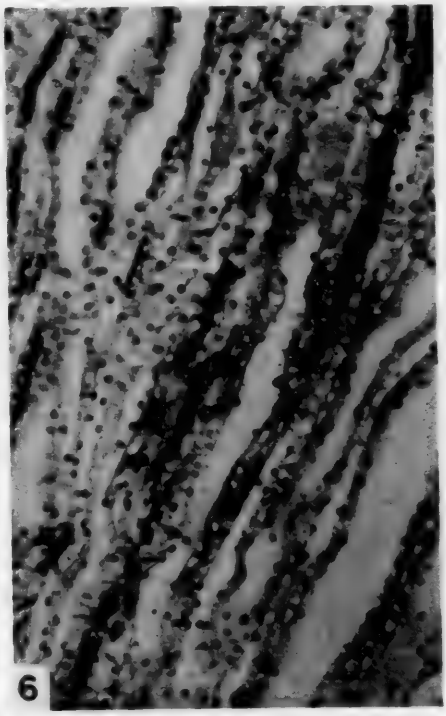
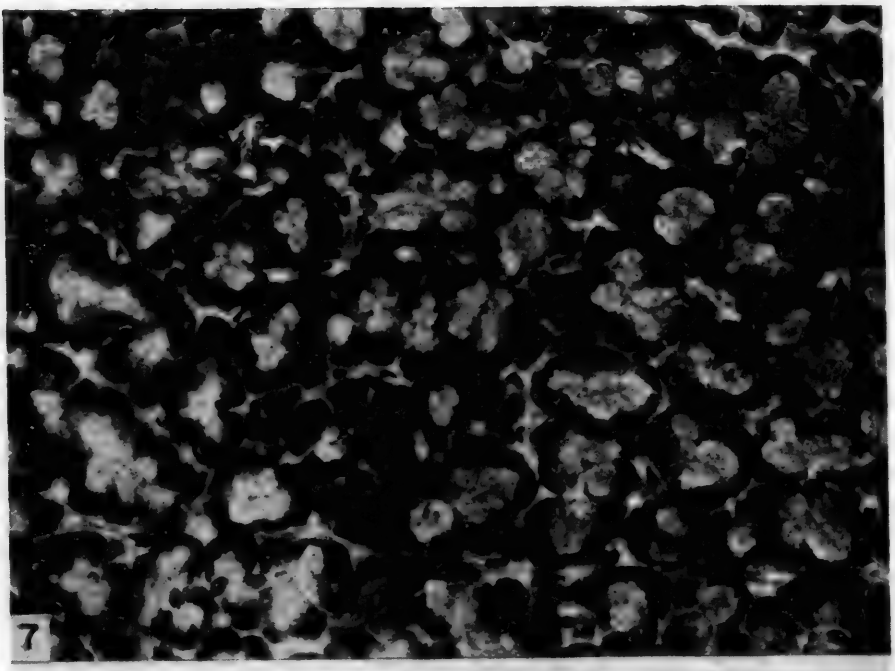


Fig. 5.—Kidney, medulla, zone between cortex and papilla. Sodium fluoride-injected animal, acute experiment. I.H.A.B. stain, ×190. Note the high degree of vascularity as evidenced by the many capillaries visible between the tubules.

Fig. 6.—Kidney, medulla, papilla. Sodium fluoride-injected animal, chronic experiment. I.H.A.B. stain, ×190. In contrast to the same area of a corresponding control (Fig. 1), note the prominence of the capillaries in this zone.

Actual adrenal cytology, both cortical and medullary, remained normal, and changes in either the content or the distribution of adrenal lipids could not be demonstrated.

Thyroid Gland.—Only the central areas of each section were studied critically because of the possibility of mechanical damage to the periphery when the gland was dissected. The follicular epithelium (in all rats of the chronic group



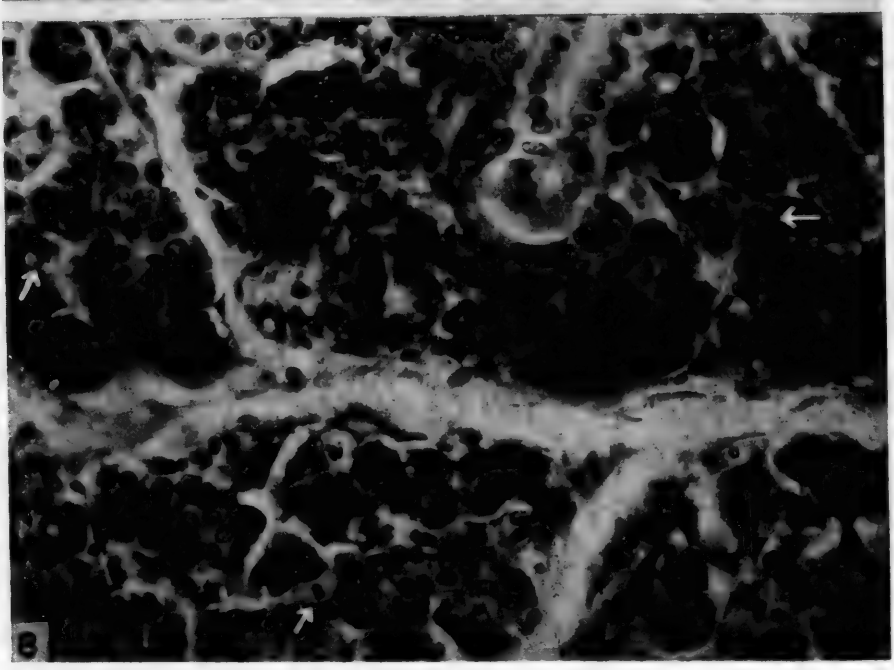


Fig. 7.—Pancreas. Control animal, chronic experiment. I.H.A.B. stain, ×400. Note the narrow connective tissue septa, the uniform character of the alveoli, and the absence of mitosis in acinar cells.

Fig. 8.—Pancreas. Sodium fluoride-injected animal, chronic experiment. I.H.A.B. stain, ×400. Note the wide connective tissue septa and the increased number of mitotic acinar cells in this experimental gland tissue as contrasted to Fig. 7. White arrows indicate examples of the mitosis.

TABLE II
MITOSES IN PANCREATIC ACINI OF RATS INJECTED WITH SODIUM FLUORIDE

EXPERIMENT	CONTROL		FLUORIDE INJECTED	
	NUMBER OF RATS	AVERAGE NUMBER OF MITOTIC NUCLEI*	NUMBER OF RATS	AVERAGE NUMBER OF MITOTIC NUCLEI
Acute	3	2.0 ± 1.42	6	2.67 ± 0.67
Chronic	3	0	5	4.8 ± 2.47

*The figures indicate the number of mitotic alveolar cell nuclei in ten high-power fields (ocular 10×, objective 40×, magnification 400×). The fields were observed in a single slide from the pancreas of each animal.

TABLE III
AVERAGE WEIGHTS OF ADRENAL GLANDS OF RATS INJECTED WITH SODIUM FLUORIDE

EXPERIMENT	GROUP	WEIGHT OF ADRENAL (MG.)*
Acute	Control	16.01 ± 0.769
	Fluoride injected	20.84 ± 1.47
Chronic	Control	18.08 ± 2.608
	Fluoride injected	23.82 ± 2.26

*Mean and standard error of weights of right adrenal gland after fixation in Bouin's solution.

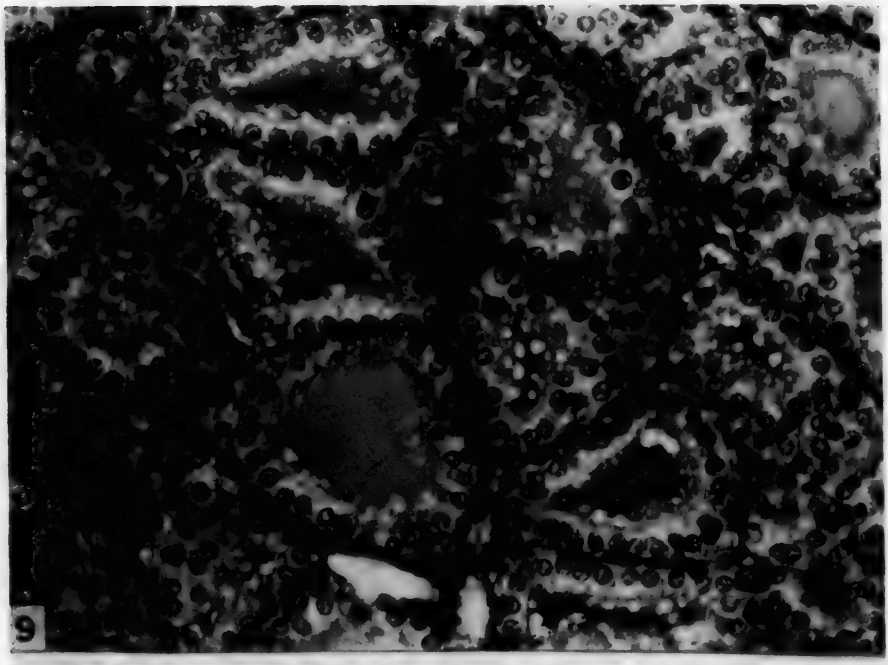
and in six out of the seven in the acute group) showed a reduction in size of the nuclei, reduction in amount of cytoplasm in the cells, and a decrease in the cell width. The follicle cells remained columnar (Figs. 9 and 10).

DISCUSSION

The normal route of fluorine excretion is through the urine.[25] Consequently, when toxic blood levels of fluoride prevail, there is selective damage to the tubular structures of the kidney by passage of the glomerular filtrate. Although the lesions here seen following intraperitoneal administration were not in all respects like those reported by other workers from feeding of the salt, the kidney was, nevertheless, the organ most severely damaged.

It would be most interesting in following the pathogenesis of fluorosis to see whether biliary excretion would rise when impairment of renal function had been attained, and whether the liver would in consequence show structural derangement to a degree approximating that developed in the kidney. Alteration of liver structure was not detected in the present study. However, the liver has been found damaged in other experiments where fluoride was fed orally, and the portal system thus placed in the line of absorption and concentration.[11, 16-18]

The observed glomerular hyperemia finds a parallel in the studies of Gottlieb and Grant,[19] and of Roholm.[5] Similarly, the hyperemia noted in the medulla may point to an initial phase of the general hemorrhage and inflammatory change summarized by Roholm.[5] Degenerations of tubular epithelium do form a part of three pathologic states which have already been mentioned and which have been consistently noted in fluorosed animals by previous investigators; i.e., subacute glomerulonephritis (chronic parenchymatous ne-



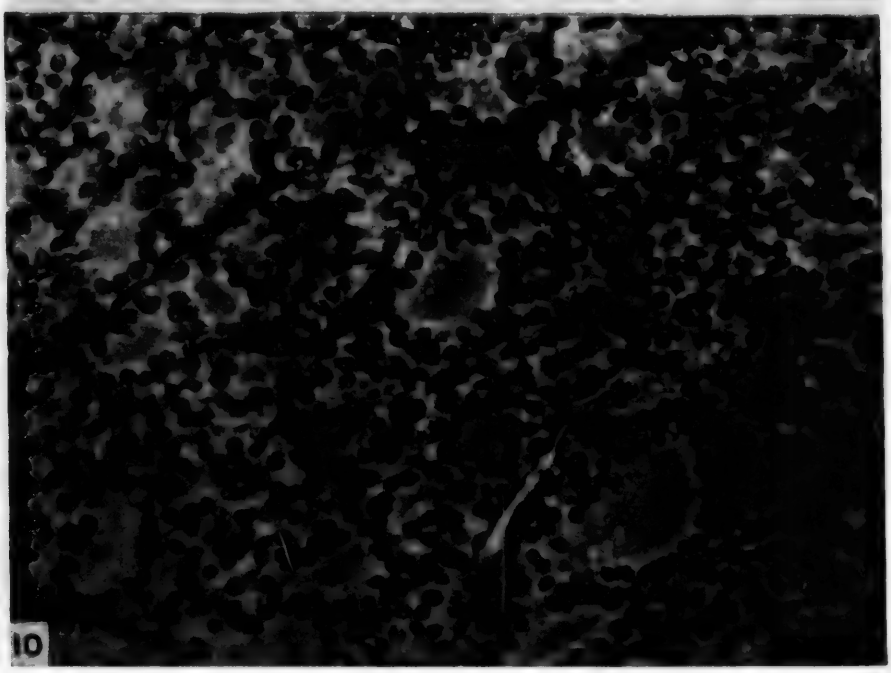


Fig. 9.—Thyroid gland. Control animal, acute experiment. I.H.A.B. stain, ×400.

Fig. 10.—Thyroid gland. Sodium fluoride-injected animal, acute experiment. I.H.A.B. stain, ×400. This section should be compared to Fig. 9. Note the following changes in the epithelial cells: (1) reduction in the size of the nuclei, (2) reduction in the amount of cytoplasm, (3) decrease in cell width and (4) closer approximation of the nuclei to one another.

phritis), chronic nephritis (chronic interstitial nephritis), and nephrosis. One might consider initial inflammation as the process which weakens the tissue and makes possible the greater breakdown which follows as the fluoride is administered chronically. Absent from the present material was the replacement of cortical parenchyma by proliferating connective tissue, such as characterizes nephritis, and the degeneration of proximal convoluted tubule epithelium found in nephrosis.[26] Instead of a diffuse effect, extending over the entire organ after the manner described by others,[5, 14, 19] the cellular changes were confined to one region of the papilla.

The alterations of pancreatic structure were in two respects similar to those detected in the parotid gland.[27] Fluoride absorption resulted, first, in a greater mitotic division of alveolar cells and, second, in wider connective tissue septa.

The adrenal cortex is known to be sensitive to conditions involving acute stress. Thus, Deanesley[28] and Ludewig and Chanutin[29] produced hypertrophy of the cortex by imposing experimental stresses. Marked decreases in stainable lipids of the cortex have been noted under similar circumstances.[28-31] The present study, however, failed to reveal change where cortical lipids were concerned. It is interesting to note that in the present study the animals of the acute group demonstrated increased adrenal weight and cortical hypertrophy accompanying fluorosis, as reported by Phillips and co-workers.[11, 16, 32] Such findings are not in agreement with those of Sollmann, Schettler and Wetzel,[15] Biester, Greenwood and Nelson,[13] and Roholm,[5] who saw no structural change in the adrenal cortex after fluorosis.

Histologic modification of the adrenal medulla after fluoride absorption was not detected. This absence of change in the medulla has been noted by all previous investigators.

The histologic changes found in the thyroid glands of treated animals were indicative of a mild atrophy. This absence of marked cellular alteration in the gland was surprising, in view of known relationships between the thyroid and the halogens. Iodine, for instance, although less active than fluorine, is concentrated to a high degree in the gland.[33] There is also the evidence of Chang and his co-workers[34] and of Phillips, English, and Hart[35] to indicate that fluorine is stored to a greater extent by the thyroid than by other organs. It would be expected that the fluoride ions, if concentrated by the gland and stored in its follicles, would have had distinct toxic effect locally. Present observations have not revealed evidence of this except for mild atrophy.

SUMMARY

1. Fluoride toxicosis was induced in young rats of the Long-Evans strain. Ten rats received a total of 406.47 mg. each of sodium fluoride injected intraperitoneally over a fifteen-day period, to induce acute poisoning. Seven rats survived, of which six were analyzed for this study. Ten others received a total of 1131.65 mg. each in 100 days, under which chronic regimen five survived.

2. Weight gain of the animals, their appearance, and the gross structure of the liver, kidney, pancreas, adrenal, and thyroid were not significantly different from those in the sodium chloride-injected controls.

3. Histologically, the kidney was the site of greatest damage and showed interstitial edema, tubular destruction, and hyperemia (glomerular and medullary). The pancreas showed increased width of connective tissue septa and evidence of increased mitosis of acinar cells. The thyroid gland was mildly atrophic. In the acutely fluorosed rats there was evidence of adrenal cortical hypertrophy, and the adrenal gland weight was increased in both groups. The liver remained histologically unchanged.

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REFERENCES

1. Jay, P., and Arnold, F. A.: Epidemiological Aspects of Oral Lactobacillus Counts in Fluoride and Non-Fluoride Areas, *J. Am. Coll. Dentists* **12**: 54, 1945.
2. Dean, H. T.: Epidemiological Studies in the United States. Dental Caries and Fluorine, Washington, 1946, American Association for the Advancement of Science.
3. Knutson, J. W., Armstrong, W. D., and Feldman, F. M.: The Effects of Topically Applied Sodium Fluoride on Dental Caries Experience, *Pub. Health Rep.* **62**: 425, 1947.
4. Ast, D. B., Finn, S. B., and Chase, H. C.: Newburgh-Kingston Caries Fluorine Study. III. Further Analysis of Dental Findings Including the Permanent and Deciduous Dentitions After Four Years of Water Fluoridation, *J.A.D.A.* **42**: 188, 1951.
5. Roholm, K.: Fluorine Intoxication, London, 1937, H. K. Lewis & Co., Ltd.
6. Pighini, G.: II, gozzo endemico a la sua etiolga-in Funzioni e disfunzioni, Milano, 1923, Publicato per cura dell'Instituto sieroterapico.
7. Goldemberg, L.: Action physiologique des fluorures, *Compt. rend. Soc. de biol.* **95**: 1169, 1926.
8. Goldemberg, L.: Action biologique due fluor, *J. de physiol. et de path. gen.* **25**: 67, 1927.
9. Christiani, H.: Altération de la glande thyroide dans l'intoxication fluorée, *Compt. rend. Soc. de biol.* **103**: 554, 1930.
10. Velu, H.: Fluore de calcium et cachexie fluorique experimentale chez le rat blanc, *Compt. rend. Soc. de biol.* **108**: 377, 1931.
11. Phillips, P. H., and Lamb, A. R.: Histology of Certain Organs and Teeth in Chronic Toxicosis Due to Fluorine, *Arch. Path.* **17**: 169, 1934.
12. May, W.: Antagonismus zwischen Jod und Fluor im Organismus, *Klin. Wchnschr.* **14**: 790, 1935.
13. Biester, H. E., Greenwood, D. A., and Nelson, V. E.: Pathological and Physiological Studies on Dogs Receiving Fluorine in Doses Comparable to Those Encountered in Some Water Supplies, *North American Veterinarian* **17**: 38, 1936.
14. Kick, C. H., Bethke, R. M., Edgington, B. H., Wilder, O. H. M., Record, T. R., Wilder, W., Hill, T. J., and Chase, S. W.: Fluorine in Animal Nutrition, *Bull. Ohio. Agric. Exp. Station* **558**: 57, 1935.
15. Sollmann, T., Schettler, O. H., and Wetzel, N. C.: Studies of Chronic Intoxication of Albino Rats, *J. Pharmacol. Exper. Therap.* **17**: 197, 1921.
16. Phillips, P. H, Hart, E. B., and Bohstedt, G.: Chronic Toxicosis in Dairy Cows Due to Ingestion of Fluorine, *University of Wisconsin Agr. Exp. Station Res. Bull.* **123**: 30, 1934.
17. Muehlberger, C. W.: Toxicity Studies of Fluorine Insecticides, *J. Pharmacol. & Exper. Therap.* **39**: 246, 1930.
18. Velu, H., and Zottner, G.: Lesions hepatique de la fluorose et de l'intoxication par les eaux phosphatées, *Compt. rend. Soc. de biol.* **109**: 354, 1932.
19. Gottlieb, L., and Grant, S. B.: Diuretic Action of Sodium Fluoride, *Proc. Soc. Exp. Biol. & Med.* **29**: 1293, 1932.
20. Schour, I., and Smith, M. C.: Experimental Dental Fluorosis, Fluorine and Dental Health, Washington, 1942, American Association for the Advancement of Science.
21. Koneff, A. A.: An Iron Hematoxylin Aniline Blue Method for Routine Laboratory Use, *Anat. Rec.* **66**: 173, 1936.
22. Bensley, C. M.: Comparison of Methods for Demonstrating Glycogen Microscopically, *Stain Technol.* **14**: 47, 1939.
23. Black, C. E.: The Staining Reactions of Fats After the Use of Various Dyes and Fixing Agents, *J. Lab. & Clin. Med.* **23**: 1027, 1937.
24. Pearl, R.: Introduction to Medical Biometry and Statistics, Philadelphia, 1940, W. B. Saunders Company, Chap. XIV, p. 340; Appendix VIII, p. 512.

25. Sollmann, T.: A Manual of Pharmacology, Philadelphia, 1948, W. B. Saunders Company, p. 804.
26. Boyd, W.: A Textbook of Pathology, Philadelphia, 1947, Lea & Febiger, p. 16.
27. Ogilvie, A. L.: Histological Findings in the Salivary Glands of the Rat Following Sodium Fluoride Administration, *J. D. Res.* **30**: 712, 1951.
28. Deanesley, R.: The Histology of Adrenal Enlargement Under Experimental Conditions, *Am. J. Anat.* **47**: 475, 1931.
29. Ludewig, S., and Chanutin, A.: Adrenal Cholesterol and Ascorbic Acid Contents After Injury, *Endocrinology* **41**: 135, 1947.
30. Robinson, F. B., and Yoffey, J. M.: Effects of Cold and Adrenaline on the Suprarenal Cortex of the Adult Male Wistar Rat, *J. Anat.* **83**: 81, 1949.
31. Jailer, J. W., and Boas, N. F.: The Inability of Epinephrine or Adrenocorticotropic Hormone to Deplete the Ascorbic Acid Content of the Chick Adrenal, *Endocrinology* **46**: 314, 1950.
32. Phillips, P. H., Lamb, A. R., Hart, E. B., and Bohstedt, G.: Studies on Fluorine in the Nutrition of the Rat. II. Its Influence Upon Reproduction, *Am. J. Physiol.* **106**: 356, 1933.
33. LeBlond, C. P.: Iodine Metabolism, Advances in Biological and Medical Physics, edited by Lawrence, J. H., and Hamilton, J. G., New York, 1948, Academic Press, p. 353.
34. Chang, C. Y., Phillips, P. H., and Hart, E. B.: The Effect of Feeding Raw Rock Phosphate on the Fluorine Content of the Organs and Tissues of Dairy Cows, *J. Dairy Sci.* **17**: 695. 1934.
35. Phillips, P. H., English, H., and Hart, E. B.: The Augmentation of the Toxicity of Fluorosis in the Chick by Feeding Dessicated Thyroid, *J. Nutrition* **10**: 399, 1935.

ROLE OF MECKEL'S CARTILAGE IN THE DEVELOPMENT AND GROWTH OF THE RAT MANDIBLE

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REPORTS on the role of Meckel's cartilage[1] in the development and growth of the mandible are conflicting. While some investigators have shown that this cartilage plays no role in mandibular growth,[1, 2] Baumüller[3] reported that the anterior section of Meckel's cartilage promotes growth of the anteromedial surface of the pig mandible. A number of studies have shown that this cartilage ossifies,[4-8] while others report that it does not undergo endochondral ossification.[9]

This disagreement in the literature concerning the fate or function of Meckel's cartilage is due to the fact that in the normal growth of bones resorption of bone tissue occurs simultaneously with apposition, and thus the growth pattern of bones is erased. Furthermore, due to the resorption, no fixed point is preserved in bones from which quantitative studies can be made. The cartilages which undergo endochondral ossification also show rapid resorption of their bony splints and a destruction of their path of growth.

In the present investigation a new method has been used. It consists of a serial histologic study of Meckel's cartilage in *ia* (incisor absent) rats in which growth of bone and cartilage occurs more or less normally, but the resorption of bone tissue is absent or severely retarded. Consequently, the perichondral bony splint which forms around Meckel's cartilage prior to, and during, its ossification persists and serves not only as an indicator of the path of growth of this cartilage, but also as a fixed point from which quantitative studies can be made. By comparing the *ia* rats at successive stages of development, the increment of bone and cartilage can be determined. Furthermore, by using certain fixed points on the *ia* Meckel's cartilages, the direction of growth of these cartilages can be ascertained. Finally, by comparing Meckel's cartilage of *ia* and normal rats at the same developmental stage, the extent of the resorption of calcified cartilage could be measured. These methods have been used previously in describing the growth pattern of the rat tibia[10] and mandible.[11]

The present investigation on the growth pattern of Meckel's cartilage showed that it not only is a center of endochondral ossification, but that it also plays an important role in the growth of the mandibular process and in the growth of the rat mandible.

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MATERIAL AND METHODS

This study is based on histologic analysis of the heads of forty-one *ia* rats and forty-three normal littermates ranging from 13 days insemination age to 30 days after birth (Table I). The heads were fixed in 5 per cent formalin, decalcified in 5 per cent nitric acid, embedded in celloidin, cut serially in frontal, longitudinal, or horizontal planes, and stained with hematoxylin and eosin. In addition to the material shown in Table I, heads of four rat embryos ranging from 19 to 21 days insemination age were fixed in Zenker-formol (5 per cent formalin), embedded in celloidin, sectioned horizontally without decalcification, and stained by von Kossa's method to ascertain the time of ossification in Meckel's cartilage.

TABLE I
THE NUMBER OF *ia* AND NORMAL RATS GROUPED ACCORDING TO AGE, THE HEADS OF WHICH WERE STUDIED HISTOLOGICALLY IN COMPLETE SERIAL SECTIONS

AGE GROUP	NUMBER OF *ia* RATS	NUMBER OF NORMAL LITTERMATES
13 days insemination age to birth	11	24
1 to 6 days	22	7
8 to 30 days	8	12
	41	43

For studying the shape of Meckel's cartilage and its relations to the surrounding structures, graphic reconstructions were made at 15, 16, 17, and 18 days insemination age stages. These reconstructions were based on the tracing of serial frontal sections 30 or 36 micra apart.

TABLE II
THE CONTRIBUTION OF ROSTRAL PROCESS IN THE ANTERIOR AND TRANSVERSE GROWTH OF THE MANDIBLE

AGE	DISTANCE FROM THE ROSTRAL PROCESS TO THE FIXED POINT ON MECKEL'S CARTILAGE (IN MICRA)	TRANSVERSE DISTANCE BETWEEN FIXED POINTS ON RIGHT AND LEFT MECKEL'S CARTILAGE (IN MICRA)
17 d-i-a*	1932	2527
18 d-i-a	2232	3047
18 d-i-a		2965
19 d-i-a	2988	3093
19 d-i-a	2868	
20 d-i-a	3792	3787
21 d-i-a	4080	3983
Birth	4080	
Birth	3930	
Birth	4220	
1 day	4200	4703
2 days	4290	
3 days	4200	4600
4 days	4680	
5 days		5080
6 days	4650	5033
8 days	4800	5733
8 days	5000	
10 days	5100	
12 days	5400	5880
15 days	6300	

*d-i-a: days insemination age.

The area of Meckel's cartilage where the perichondral splint first forms and the ossification center first appears was preserved in the *ia* rats. This area was used as a fixed point from which various measurements were made (Table II).

OBSERVATIONS

A. Prior to the Establishment of the Squamomandibular Articulation (13 to 19 Days Insemination Age).—

1. *Differentiation of Meckel's cartilages and rostral process:* At 13 days insemination age, the caudal boundary of the primitive oral cavity of the rat embryo is formed by the mandibular processes. On both the right and left sides, in about the middle of the mandibular process, the mesenchyme undergoes condensation and differentiation into prechondrocytes (Fig. 1). These are the anlagen of the right and left Meckel's cartilages (Fig. 3). From this central area in the mandibular process the anterior end of the anlage of each Meckel's cartilage grows forward and inward, and at 16 days insemination age it fuses in the midline with the opposing anlage to form a structure which has been termed the rostral process[11] (Fig. 4). The posterior end of the anlage of each Meckel's cartilage grows backward and outward, and at 16 days insemination age it forms a hook-shaped process just lateral to the developing otic capsule. This is the anlage of the malleus and incus (Fig. 4). At 16 days insemination age the prechondrocytes in the anlage of Meckel's cartilage differentiate into true chondrocytes.

Thus, at 16 days insemination age, the cartilaginous skeleton of the mandibular process consists of two Meckel's cartilages which extend downward, forward, and inward from the otic capsule and meet each other to form the rostral process at the anterior end of the mandibular process (Figs. 2 and 4). These cartilages, at this time, are the support by which the mandibular process is held to the developing neurocranium.

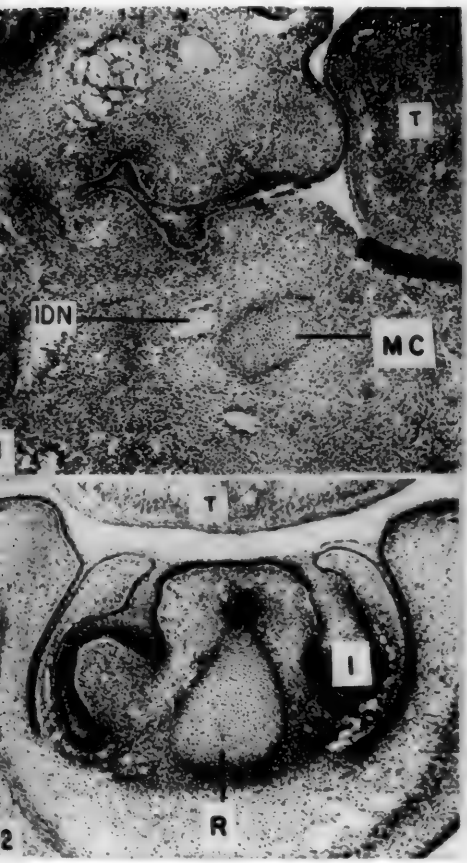
Between 15 and 16 days insemination age in the area of the mandibular process where the anlage of Meckel's cartilage first formed, the mandible ossifies as a plate of bone lateral to the cartilage[11] (Fig. 4). This plate of bone grows to form a tube of bone, and at 17 days insemination age the ramus and the body of the mandible develop.[11] The relations between the mandible and Meckel's cartilage are as follows: the bony tubes and plates of the body of the mandible surround the rostral process and Meckel's cartilage (Fig. 7), while the ramus develops lateral to the cartilage.[11]

2. *Growth of Meckel's cartilages and the mandibular process:* Meckel's cartilages grow at their posterior ends, at their anterior ends in the rostral process, and along their circumference.

The growth at the posterior ends is both appositional and interstitial, and it is directed upward, backward, and outward. This direction of growth keeps pace with the widening neurocranium, and also results in a downward and forward shift of the mandibular process.

The growth at the anterior ends occurs almost exclusively in the rostral process and is both appositional and interstitial. At the anterior end of the

rostral process, the appositional growth progresses by differentiation of the perichondral mesenchymal cells into prechondrocytes, and later into chondrocytes. Interstitial growth occurs in a central proliferative zone in the rostral process where the chondrocytes divide in two planes. Right and left planes of division are at an angle of about 45 degrees to the midsagittal plane. As a result of this directional proliferation, columns of daughter cells can be seen directed laterally and posteriorly, continuing from the rostral process into right and left Meckel's cartilage (Fig. 5). The existence of a center of longitudinal growth at the anterior ends of Meckel's cartilages and in the rostral process is related to the simultaneous growth in length and width of the head in general, and of the mandibular process in particular. Any increment at anterior ends will cause a lateral shift of all posterior parts of Meckel's



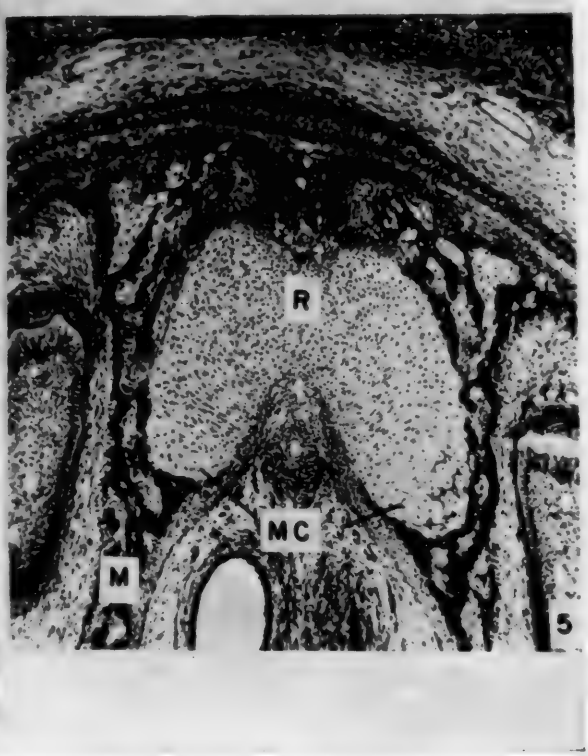
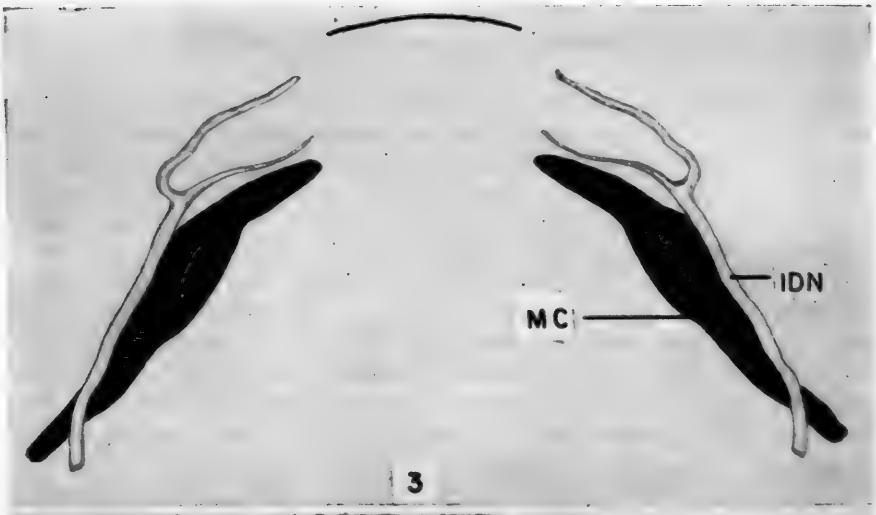
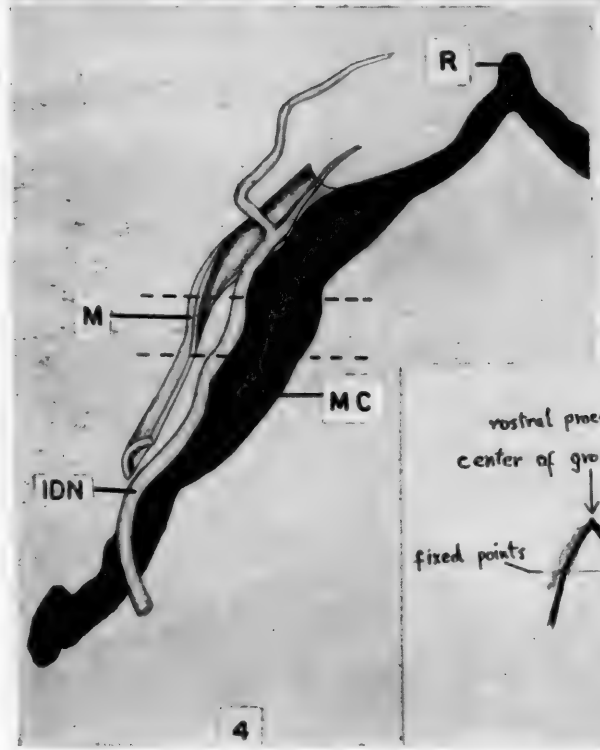


Fig. 1.—Frontal section through middle of mandibular process in head of a rat embryo 13 days insemination age. Note Meckel's cartilage anlage (*MC*). *IDN*, Inferior dental nerve; *T*, tongue. ×70.

Fig. 2.—Frontal section through anterior part of mandibular process in rat embryo 17 days insemination age. Note the rostral process (*R*) in midline. *I*, Incisor tooth germs; *T*, tongue. ×70.

Fig. 5.—Horizontal section through part of rostral process (*R*) and right and left Meckel's cartilages (*MC*). ×70.





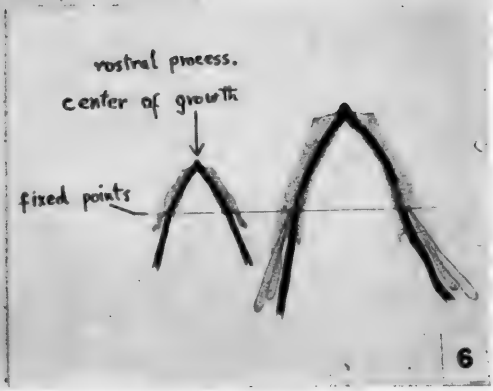


Fig. 3.—Graphic reconstruction, seen from above, of Meckel's cartilage (*MC*) and inferior dental nerve (*IDN*) in rat embryo 15 days insemination age. Note that Meckel's cartilages on right and left sides do not meet at their anterior ends as yet. Black line near top indicates the anterior boundary of the mandibular process. ×83.

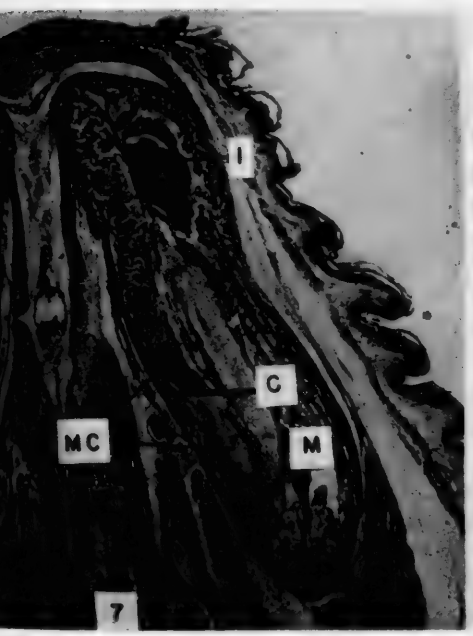
Fig. 4.—Graphic reconstruction, seen from above, of left Meckel's cartilage (*MC*), inferior dental nerve (*IDN*), and first plate of bone of mandible (*M*) in rat embryo 16 days insemination age. Note that the right and left Meckel's cartilages meet at their anterior ends to form the rostral process (*R*). Area between broken lines indicates the region of mandibular ossification center and the region where Meckel's cartilage anlage first differentiates.

Fig. 6.—Diagram, based on actual measurements, to show how growth in rostral process serves both to lengthen the mandible and to increase the transverse distance between two fixed points on right and left sides. Diagram on left represents the mandible at 18 days insemination age, that on right at 4 days of age. Horizontal line passes through the fixed points which were preserved in the *in* rats. Meckel's cartilage and rostral process are shown in black, stippled area represents the outline of the mandibles. ×45.

cartilage and related structures. This mode of growth results, therefore, not only in the forward growth, but also in an increase of the transverse distance between the right and left sides of the mandibular process and the mandible (Fig. 6).

The circumferential growth of Meckel's cartilages is mainly appositional and occurs along their entire length.

B. After the Formation of Squamomandibular Articulation (20 Days Insemination Age to 30 Days After Birth).—At 19 days insemination age the articulation between the mandible and the skull begins to be established.[11] Starting at 20 days insemination age, the part of Meckel's cartilage which lies anterior to the mandibular ossification center begins to undergo endochondral ossification (Fig. 7). One day later the part which lies posterior to the mandibular ossification center starts to degenerate (Fig. 7).



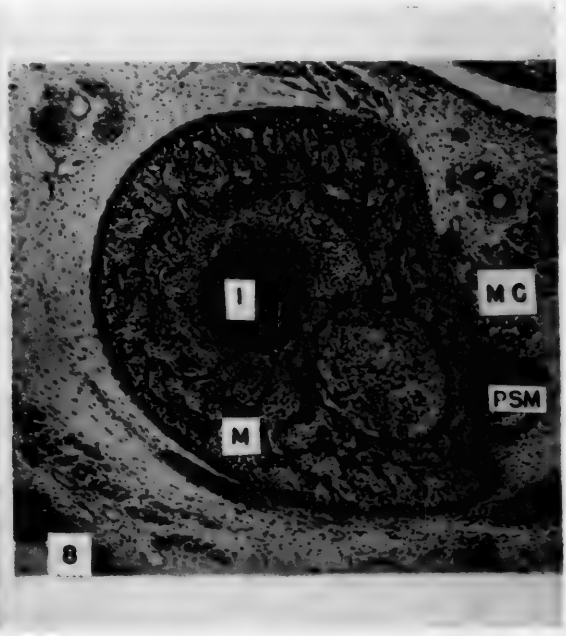
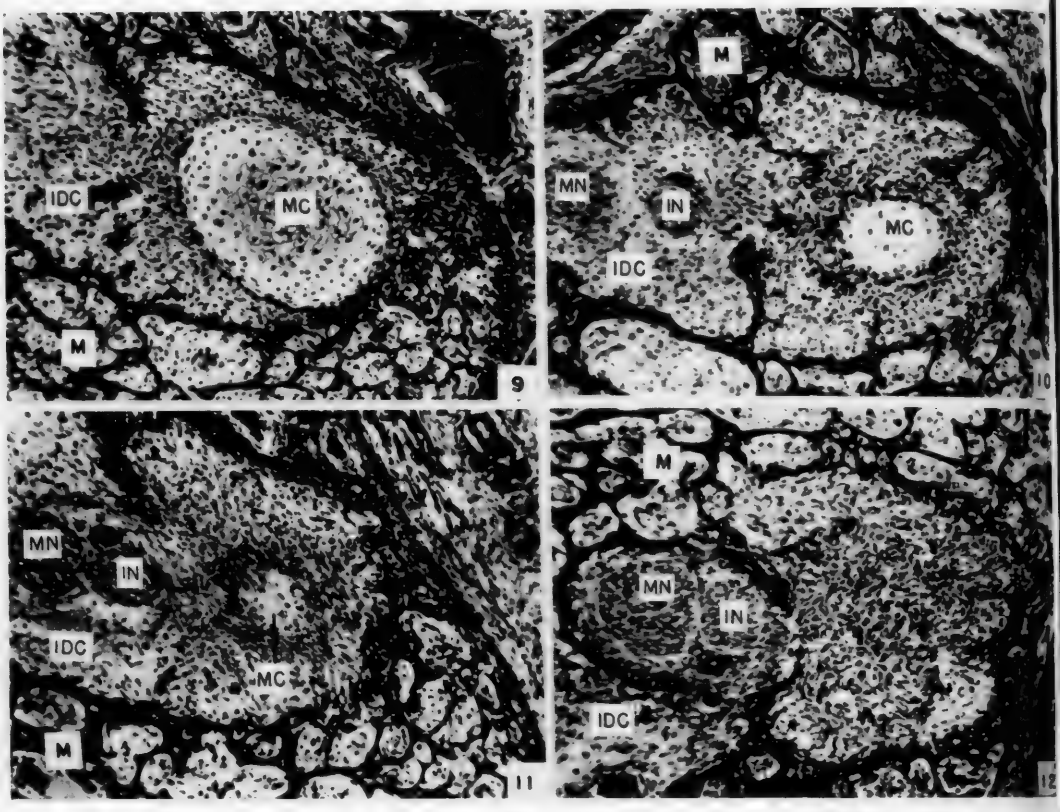


Fig. 7.—Horizontal section through the right mandible of a rat 21 days insemination age. Note that the part of Meckel's cartilage anterior to line *C* is undergoing endochondral ossification. Part posterior to line *C* is not calcified and has begun to degenerate. *MC*, Meckel's cartilage; *M*, mandible; *I*, incisor tooth germ. ×21.

Fig. 8.—Frontal section through the anterior part of mandible (*M*) and Meckel's cartilage (*MC*) at 20 days insemination age. Note the perichondral splint (*PSM*) around Meckel's cartilage and erosion of chondrocytes. Note how the mandible surrounds the cartilage. *I*, Incisor tooth germ. ×70.

The endochondral ossification of the part of Meckel's cartilage anterior to the mandibular ossification center follows the sequence of changes as has been described for endochondral ossification of tubular bones. Around the cartilage a perichondral splint forms, the chondrocytes undergo edematous changes, and the intercellular cartilaginous matrix calcifies. Erosion of the calcified cartilage and its invasion by young mesenchyme occur simultaneously (Fig. 8). On



Figs. 9, 10, 11, and 12.—Frontal sections through the region of ossification center of mandible at 20 days insemination age, 21 days insemination age, 1 day after birth, and 3 days of age, respectively. Note the progressive degeneration of Meckel's cartilage in this region and its replacement by connective tissue and bone. *M*, Bony plates of mandible; *MC*, Meckel's cartilage; *IDC*, inferior dental canal; *MN*, mental nerve; *IN*, incisive nerve. ×150.

the remnants of calcified cartilage, bone is laid down. The formation of the perichondral splint and the process of endochondral ossification finally extend into the rostral process. While these changes progress anteriorly, directional growth continues in the rostral process.

The process of degeneration of the posterior portion of Meckel's cartilage begins in the area of the mandibular ossification center (Fig. 7). The chondrocytes show pyknotic nuclei; the diameter of Meckel's cartilage becomes smaller, and in the surrounding mass of cells macrophages can be distinguished. At 1 day of age Meckel's cartilage in the region of the mandibular ossification center is reduced to a thickness of only three or four chondrocytes, and at 3 days of age the cartilage in the area of the mandibular ossification center is completely destroyed and replaced by connective tissue. In the connective tissue which replaces the cartilage, fine mosaic-like trabeculae of bone appear (Figs. 9 to 12).

Thus, at 3 days Meckel's cartilage is split into two parts: the anterior part, including the rostral process which undergoes ossification, and a pos-

terior part which begins to degenerate. The subsequent changes in these two portions of the cartilage will be considered separately.

1. *Part showing endochondral ossification:* From 3 to 30 days of age, the part of Meckel's cartilage anterior to mandibular ossification center continues to grow at the rostral process. As Meckel's cartilages and their rostral process grow anteriorly, the bony splints surrounding them also grow. Calcification of cartilage matrix, resorption of the calcified matrix, and its replacement by bone progress in the same direction. The directional growth in the rostral process continues to cause a forward growth of Meckel's cartilages and an increase in the transverse distance between the right and left cartilages. Since these cartilages are surrounded by the right and left mandibles, the growth in the rostral process guides the anterior growth of the mandible and widens the distance between right and left mandibles (Fig. 6). With increasing age the plane of division of the rostral chondrocytes shifts progressively toward the sagittal plane. As a result of this change in directional proliferation, the distance between right and left mandibles increases more markedly than the growth in the anterior direction.

Up to 3 days the rostral process was oval in cross section, with its long axis in a horizontal plane (Fig. 13). Between 3 and 6 days, a comblike cartilaginous crest forms on its superior surface which imparts to the cross section an approximately triangular shape (Fig. 14). Between 8 and 10 days the lower part of the rostral process begins to degenerate and is replaced by connective tissue (Fig. 15). The cells in this area assume a stellate appearance, and, within the homogeneous matrix, fibrils now can be seen. It seems likely that this replacement of the cartilage by fibrous tissue is due to the differentiation of younger cells into fibroblasts instead of chondrocytes. Between 10 and 12 days of age the inferior part of the rostral process is completely replaced by connective tissue (Fig. 16). The superior part persists as a plate of hyaline cartilage which separates the anterior parts of the right and left mandibles. It consists of five zones of cells. In a narrow central zone the cells are flattened and arranged with their long axes in the sagittal plane. Lateral to the central zone, there is on either side a zone with ovoid or spherical cells which form horizontal columns. Lateral to these zones, the intercellular substance calcifies and the replacement of cartilage by bone occurs (Fig. 16). Between 15 and 19 days of age the rostral process gradually changes from hyaline to fibrocartilage. This plate of fibrocartilage persisted in the oldest specimen studied (30 days).

2. *Part undergoing degeneration:* At 3 days of age, this part of Meckel's cartilage extends backward from the mandibular ossification center. In its backward course it is surrounded by the molar region of the mandible, while farther back it lies medial to the ramus. From there it extends backward and upward to the base of the skull where it lies dorsal to the anterior process of the malleus (goniale) (Fig. 17). Still farther back, Meckel's cartilage is related to the middle ear, and there it ends in two endochondral bones, the malleus and the incus.

The degeneration of this part of Meckel's cartilage and its replacement by connective tissue progresses backward from the region of the mandibular ossification center. At 6 days the entire part of cartilage which was surrounded by the molar region of the mandible is replaced by connective tissue.

Between 3 and 6 days, degeneration of Meckel's cartilage and its replacement by connective tissue begin in a second area, which is located in the region of the anterior end of the goniale. Here the entire thickness of Meckel's cartilage is replaced by connective tissue (Figs. 17 to 19). This process advances posteriorly toward the otic part of the cartilage. Thus, at 6 days the part of the cartilage that is related to the mandible is separated from the part related to the middle ear. At 6 days the part related to the mandible extends from

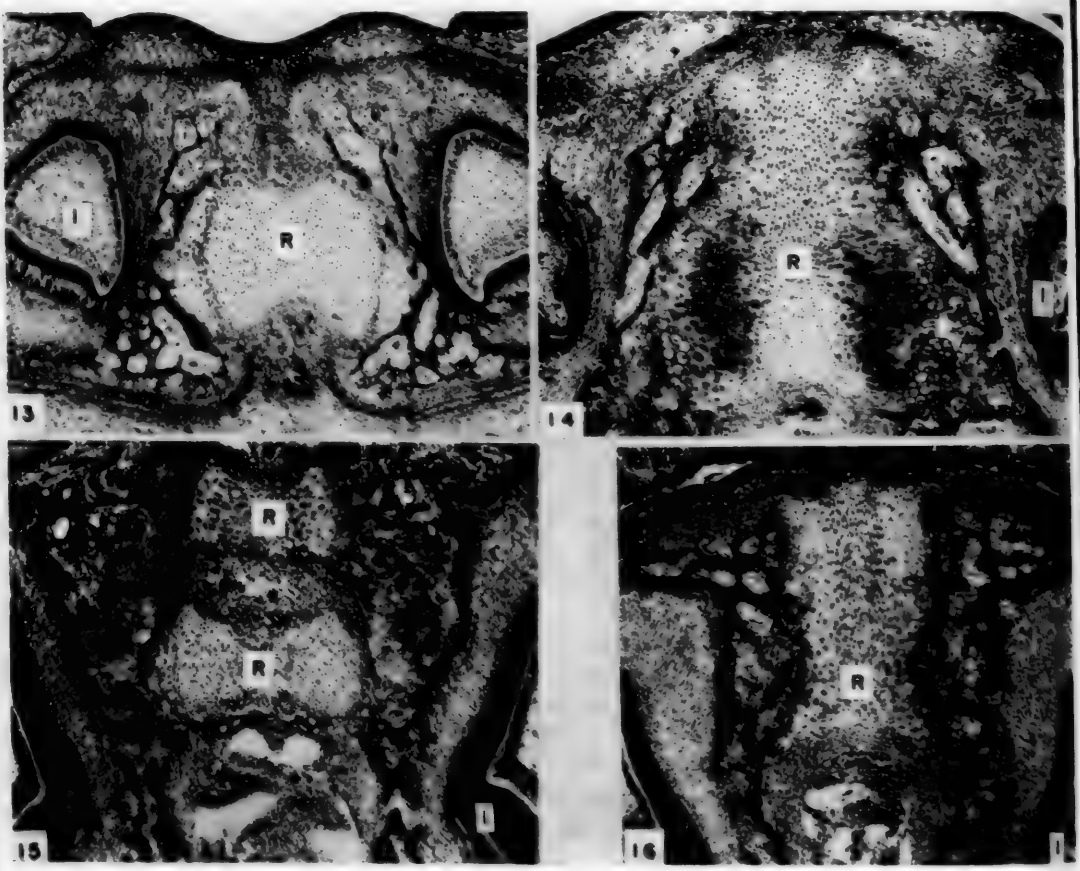


Fig. 13.—Frontal section through rostral process (*R*) at 2 days of age. *I*, Incisor tooth germ. ×70.

Fig. 14.—Frontal section through rostral process (*R*) at 6 days. Note the formation of a cartilaginous crest on the superior surface of rostral process. ×70.

Fig. 15.—Frontal section through rostral process (*R*) at 10 days. Note that the lower part of rostral process is being replaced by connective tissue. ×70.

Fig. 16.—Frontal section through rostral process (*R*) at 12 days. Note that the lower part of rostral process is completely replaced by connective tissue. ×70.

Figs. 17, 18, and 19.—Frontal sections through Meckel's cartilage in area where the cartilage lies inferior to the cranial base at 3, 5, and 6 days, respectively. Note the progressive degeneration of cartilage and its replacement by connective tissue. This split separates the otic and mandibular parts of Meckel's cartilage. *MC*, Meckel's cartilage; *d*, condule, or the anterior process of malleus; *TY*, tympanic bone. ×70.

the level of the mandibular ramus to the base of the skull. The degeneration of this part of Meckel's cartilage progresses from front backward, and at 17 days of age it is completely replaced by connective tissue. The connective tissue which replaces this cartilage does not form the sphenomandibular ligament.

C. Quantitative Study of the Growth in the Rostral Process.—Since, in the *ia* mandibles, various fixed points were available, quantitative measurements were made to determine the effect of directional proliferation in the rostral process on the anterior and transverse growth of the mandible. The perichondral splint which first formed around Meckel's cartilage, and which also corresponds to the area where the mandible first begins to ossify, was used as a fixed point. Measurements were made from this point to the anterior end of the rostral process at successive stages. These measurements are given in Table II, and they show the contribution of rostral growth in the anterior increments of the mandible. From 17 days insemination age to 15 days after birth the part of the mandible anterior to its ossification center grows from about 2 mm. to about 6.5 mm.

Measurements were also made at successive stages from one fixed point in the right mandible to the same fixed point in the left mandible. These measurements are shown in Table II, and they indicate the effect of rostral growth on the width of the mandibular arch. The transverse distance between the ossification centers on the right and left sides increases from 2.5 mm. at 17 days insemination age to about 6 mm. at 12 days after birth.

DISCUSSION

Since the first description of Meckel's cartilage,[1] no detailed report dealing with its growth, function, or fate has appeared in the literature. Investigators studying the development of the mandible in various mammals have referred to it only briefly, and are in disagreement regarding its role and final fate. Some investigators have claimed that it does not ossify and that it plays no role in the development of the mandible.[1, 9] Others have shown that it ossifies in part, but believe that it does not contribute to mandibular development or growth.[2] The descriptions by different authors vary according to the developmental period to which they confined their investigations.

The present study has shown that Meckel's cartilage plays an important role in the growth of the mandibular process, and later of the mandible of the rat. Its function is twofold. It supports and directs the growth of the mandibular process prior to the ossification of the mandible and the establishment of the articulation of this bone with the brain case; and after the mandible ossifies, Meckel's cartilage directs and aids the growth of this bone in anterior and transverse directions. The present observations show that Meckel's cartilage in the rat does not completely disappear, but even at 30 days of age persists as a plate of fibrocartilage which is derived from the rostral process.

Up to 21 days insemination age, although a large part of the bony skeleton of the mandible has formed, its articulation with the skull is not established. Up to this stage, Meckel's cartilage is the only support of the mandibular process and the only means by which the mandible is attached to the neuro-

cranium. Meckel's cartilage is the main support of the mandibular process in much the same way as the cartilaginous models of the tubular bones are the supports of the growing limb buds. It not only serves as the skeleton around which the nerves and muscles can orient themselves, but also directs the growth of the mandibular process. Proliferation of the chondrocytes in the rostral process is directional and so arranged that it causes both lengthening and widening of the mandibular arch. Posterior and superior growth at the otic or cranial ends of Meckel's cartilage causes lengthening and caudal shift of Meckel's cartilage. At this stage, therefore, the posterior otic part of Meckel's cartilage serves precisely the same function which later is performed by the condylar cartilage.

After the articulation between the squamosal and the mandible has been fully established (21 days insemination age), degeneration of Meckel's cartilage begins posterior to the mandibular ossification center. At 3 days after birth, the cartilage is divided into two histologically and functionally different parts. The posterior part is embedded in the molar region of the mandible and undergoes gradual degeneration, which progresses in a backward direction. However, the anterior part undergoes endochondral ossification. Continued directional growth in the rostral process causes lengthening of the mandible in an anterior direction and widening of the distance between the two halves. With age, the direction of growth in the rostral process changes so that it contributes mainly to the widening and less to the lengthening of the mandible.

The rostral process, in addition to being a growth center, acts as a symphysis between the two halves of the mandible. It consists of hyaline cartilage only up to the time when the teeth come into function. At 19 days after birth, the rostral process is replaced by fibrocartilage. Around this time the rat begins to eat hard food (weaning age 21 days). Mobility of the two halves of the mandible at the symphysis is an important characteristic of mastication in the rat. Replacement of hyaline by fibrocartilage seems to be correlated with the masticatory function.

SUMMARY

This study is based on a serial histologic investigation of heads of 41 *ia* and 43 normal rats ranging in age from 13 days insemination to 30 days after birth. The findings were:

1. Right and left Meckel's cartilages begin to differentiate independently in the branchial mesenchyme at 13 days insemination age. They grow forward and inward to meet in the midline to form the rostral process.

2. Prior to the formation of the squamomandibular articulation, growth at the posterior end of Meckel's cartilages serves to cause a downward and forward shift of the mandibular process and the mandible. Growth in the rostral process serves to cause a forward growth of the mandibular process and mandible, and to widen the distance between right and left mandibles.

3. After the formation of the squamomandibular articulation, the posterior part of Meckel's cartilage degenerates but does not form the sphenomandibular

ligament. The anterior part undergoes endochondral ossification and serves to cause a forward growth of the right and left mandibles, and to increase the transverse distance between them.

4. The otic part of Meckel's cartilage is separated from the mandibular part at 6 days of age.

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REFERENCES

1. Meckel, J. F.: Handbuch der menschlichen Anatomie, Bd. IV. Halle u. Berlin, 1820.
2. Low, A.: Further Observations on the Ossification of the Human Lower Jaw, *J. Anat. & Physiol.* **44**: 83, 1909.
3. Baumüller, B.: Ueber die letzten Veränderungen des Meckelschen knorpels, *Ztschr. wiss. Zool.* **32**: 466, 1879.
4. Callander, G. W.: The Formation and Early Growth of the Bones of the Human Face, *Phil. Tr. Roy. Soc., London* **159**: 163, 1869
5. Fawcett, E.: Ossification of Lower Jaw in Man, *J. A. M. A.* **45**: 697, 1905.
6. Low, A.: The Development of the Lower Jaw in Man, *Proc. Anat. Soc. Gr. Britain a. Ireland; J. Anat. & Physiol.* (new series) **9**: 1905.
7. Stieda, L.: Studien über die Entwicklung der Knochen und des Knochengewebes, *Arch. f. mikr. Anat.* **11**: 235, 1875.
8. Strong, R. M.: The Order, Time, and Rate of Ossification of the Albino Rat (Mus Norveigicus Albinus), *J. Anat.* **36**: 313, 1925.
9. Brock, J.: Ueber die Entwicklung des Unterkiefers des Säugethiere, *Ztschr. wiss. Zool.* **27**: 258, 1876.
10. Bhaskar, S. N., Weinmann, J. P., Schour, I., and Greep, R. O.: The Growth Pattern of the Tibia in Normal and *ia* Rats, *Am. J. Anat.* **86**: 439, 1950.
11. Bhaskar, S. N.: Growth Pattern of the Rat Mandible From 13 Days Insemination Age to 30 Days After Birth, *Am. J. Anat.* **92**: 1, 1953.

ON THE DEVELOPMENT OF DENTIN IN FISH

II. Teleostei

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INTRODUCTION

IN MOST of the bony fish, the tips of the teeth consist of a substance which is so densely calcified that its organic matrix is destroyed during routine decalcification. The development of this substance, which has been called mesodermal enamel, was described in a previous dissertation.[3]

In the developmental stages, the organic foundation of the mesodermal enamel in Teleostei consists of collagenous fibrils and is laid down by the dental papilla. In some species, the collagenous fibrils are formed as precollagenous, argyrophil fibrils which are subsequently transformed into collagenous fibrils. In other species, the collagenous fibrils are formed without any argyrophil prestage, but wholly or partly as fibroglia fibrils in the cytoplasm of the peripheral papilla cells.

The development of the dentin takes place inside and basally to the mesodermal enamel. The investigation of dentin development is based on the same material as was used earlier in studying the development of the mesodermal enamel.[3]

MATERIAL AND METHODS

In the bony fish, new teeth are developed during the entire life of the fish as the old ones fall out. After the teeth have started to form, we can therefore find, in most cases, tooth germs in all stages of development in the same specimen.

The material comprises the following species: *Labrus bergylta Ascanius, Ctenolabrus rupestris Linnaeus, Merluccius merlucius Linnaeus, Anguilla vulgaris Linnaeus,* and *Gobius niger Linnaeus.* In the case of *Merluccius merlucius,* only the development of the outer area, but not the inner vasodentin, has been investigated.

The treatment of the material in preparing the jaws, fixation, decalcification, embedding, sectioning, and staining was the same as in the case of *Squalus acanthias.*[4]

OBSERVATIONS

The formation of the dentin of the various species selected commences after the foundation of the mesodermal enamel has been deposited. In certain of these, the dentin formation has started at a time when the organic founda-

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tion of the mesodermal enamel can be readily preserved (Fig. 1). In other species, the calcification of the mesodermal enamel has advanced so far that large parts are lost in routine decalcification of the sections before the formation of the dentin begins (Fig. 2).

The first sign of dentin formation in the bony fishes studied occurs near the basal edge of the ectodermal part of the tooth germ which grows into the mesoderm. In some of the species, the epithelium penetrates so deeply into the mesoderm that nearly the whole length of the tooth is established before any appreciable amount of dentin is formed (Fig. 2). This condition was noted with particular frequency in *Anguilla vulgaris.*

Fig. 1.

Fig. 2.

Fig. 1.—*Gobius niger.* Tooth germ with initial dentin development. Wilder's silver impregnation, Pasini-Walter. ×360. *tep,* Tooth germ's ectodermal part; *me,* mesodermal enamel; *dp,* dental papilla; *id,* initial dentin development.

Fig. 2.—*Anguilla vulgaris.* Tooth germ with initial dentin development. Wilder's silver impregnation, azan. ×360. *me,* Mesodermal enamel; *d,* dentin; *kf,* von Korff's fiber; *id,* initial dentin development.

During this increased deepening of the epithelium, the membrana terminans is distinctly seen and can often be followed from the outside of the tooth germ's ectodermal part, around the basal edge of the tooth germ, and to the point where it passes over to the organic foundation of the mesodermal enamel (Fig. 1).

In *Anguilla vulgaris,* early dentin formation is different from other species in that along the sides of the dental papilla, where the ectodermal part is growing rapidly, von Korff's fibers are found for the whole extent of the membrana terminans. These fibers extend inward from the membrane toward a central fiber plexus in the papilla (Fig. 2). They can be found in late stages of dentin formation, but are not so numerous as in the earlier phases of dentin development.

In the other species examined, it can often be observed that the fibers in the membrana terminans basally to the mesodermal enamel lie more or less longitudinally to the tooth, and often in undulations (Fig. 1). At the base of the tooth germ, large numbers of argyrophil fibers extend from the membrana terminans over into the mesoderm. In certain cases, they begin fanwise in

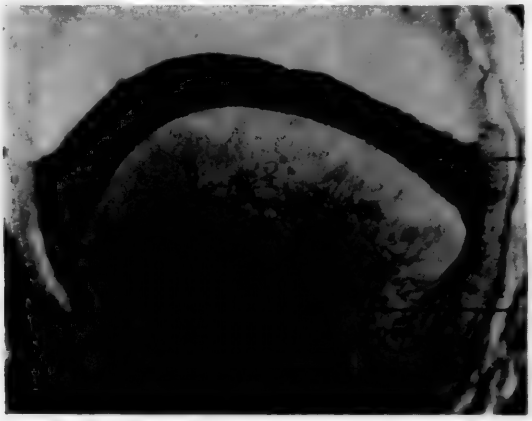

Fig. 3.—*Labrus bergylta.* Tooth germ with initial dentin development. Wilder's silver impregnation, azan. ×360. *d*, Dentin; *dp*, dental papilla.

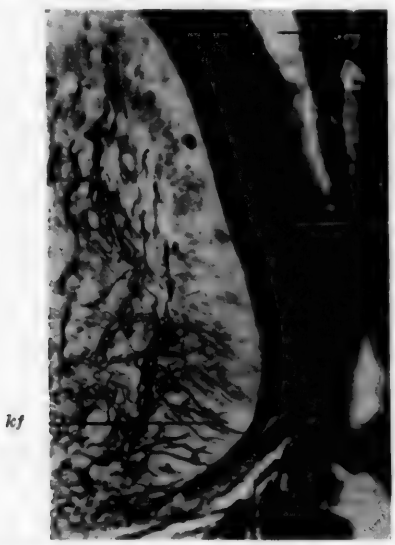

Fig. 4.—*Labrus bergylta.* Division of tooth germ with dentin development in progress. Wilder's silver impregnation, hematoxylin, fuchsin. ×360. *me*, Mesodermal enamel; *lf*, longitudinal dentinal fibers; *rf*, radial dentinal fibers; *kf*, von Korff's fiber.

the membrane (Fig. 3). In other cases, they occur individually and are more perpendicular to the direction of membrana terminans, but join with the membrane (Fig. 4). It is especially in the first stages of dentin formation that

argyrophil fibers pass from the dentin to the dental papilla (Fig. 7). As the dentinal layer widens, these fibers are only rarely seen.

In *Merluccius merlucius*, the whole dentinal layer appears to be built up by fibers which extend longitudinally in the same direction as the fibers in membrana terminans (Figs. 6 and 7). All the fibers are very coarse and ap-

Fig. 5.—*Gobius niger*. Tooth germ with dentin development in progress. Wilder's silver impregnation, azan. ×360. *tep*, Tooth germ's ectodermal part; *d*, dentin; *dp*, dental papilla.

Fig. 6.

Fig. 7.

Fig. 6.—*Merluccius merlucius*. Tooth germ with dentin development in progress. Wilder's silver impregnation, Pasini-Walter. ×360. *tep*, Tooth germ's ectodermal part; *d*, dentin; *dp*, dental papilla.

Fig. 7.—*Merluccius merlucius*. Division of tooth germ with the latest formed dentin. Wilder's silver impregnation, Pasini-Walter. ×810. *tep*, Tooth germ's ectodermal part; *d*, dentin; *dp*, dental papilla; *mt*, membrana terminans with papilla fibers running into it.

Fig. 8.

Fig. 9.

Fig. 8.—*Gobius niger.* Tooth germ with dentin development in progress. Wilder's silver impregnation, Pasini-Walter. ×360. *tep*, Tooth germ's ectodermal part; *rf*, radial dentinal fibers; *dp*, dental papilla; *lf*, longitudinal dentinal fibers.

Fig. 9.—*Gobius niger.* Same section and the same lettering used as in Fig. 8. ×600.

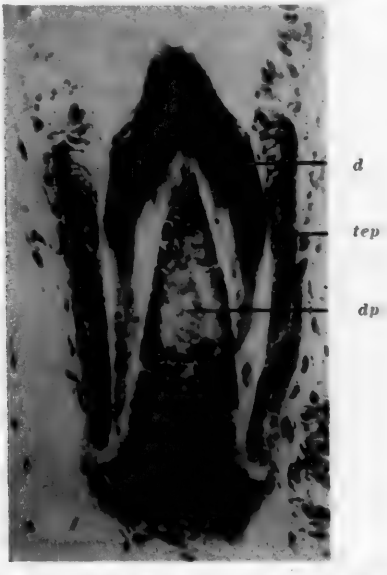

Fig. 10.—*Gobius niger.* Tooth germ with dentin development in progress. Iron hematoxylin, picro-fuchsin. ×360. *d*, Dentin; *tep*, tooth germ's ectodermal part; *dp*, dental papilla.

pear to be continuous. While the fibers in membrana terminans seem to lie very closely packed together, the others are separated.

In *Gobius niger, Labrus bergylta,* and *Ctenolabrus rupestris,* the initial fiber organization is similar to *Merluccius merlucius* (Figs. 4, 8, and 9). At the base of the dental papilla, where dentin formation has just begun, these fibers lie close to the columnar epithelium of the tooth germ (Fig. 4). Below the mesodermal enamel cap, a second dentinal layer is present between the longitudinally oriented dentinal fibers and the columnar epithelium. This layer gradually tapers off toward the basal part of the tooth germ, and has transverse fibers which are finer than the longitudinal fibers (Fig. 9).

At this stage, dentin formation internal to the mesodermal enamel has just begun. This dentin is initiated without the presence of a membrana terminans at the boundary of the mesodermal enamel and the dental papilla, and only rarely with the presence of von Korff's fibers (Figs. 1 and 2). Often there is a very close system of argyrophil fibers in the whole of the central portion of the dental papilla (Figs. 3 and 5), but at some distance from the dentin the fibers terminate rather abruptly. In the outermost area of the dental papilla, they are absent.

In a few rare cases, especially in that of *Anguilla vulgaris,* a number of von Korff's fibers are visible during the first dentin formation just beneath the mesodermal enamel (Fig. 2).

Observed conditions in *Gobius niger* differed from other species (Fig. 10). Along the periphery of the dental papilla, there is a series of cell nuclei close to the dentin. From the center of the dental papilla, basally, the cells are closely placed. The rest of the papilla has a granular appearance and resembles cytoplasm.

DISCUSSION

From these investigations, there does not seem to be any uniform system of development for the dentin in bony fish.

When the dentin development starts in *Anguilla vulgaris,* von Korff's fibers can be traced from the dental papilla into the dentin. These fibers extend along the sides of the papilla where a membrana terminans already forms the outer boundary of the dentin, and also along the inside of the mesodermal enamel (Fig. 2). The von Korff fibers seem to become embedded in the dentin as this grows in thickness. However, there seems to be too few von Korff fibers to form the entire dentinal matrix.

In *Merluccius merlucius* one gets the impression that the membrana terminans is the origin of most of the dentinal matrix fibers (Figs. 6 and 7). The fact that these fibers are more widely dispersed than in the early membrana terminans may indicate that the growth of the dentinal layer may be due both to additive stratification of fibers from the dental papilla and to a dispersion of the fibers, such that they occupy a greater space.

In *Gobius niger, Labrus bergylta,* and *Ctenolabrus rupestris,* it is presumed that the development of the longitudinal fibers in the dentin is similar to that

in *Merluccius merlucius* (Figs. 4, 8, and 9). In these species, transverse dentinal fibrils are found external to the longitudinal fibers. They apparently form secondarily and displace the longitudinal fibers inward toward the dental papilla. It is also conceivable that the transverse fibrils, during their formation, might displace the ectodermal part of the tooth germ outward, with the result that the tooth becomes thicker than when first initiated.

In all the species investigated, except for *Anguilla vulgaris*, von Korff's fibers are found only during the development of the outermost dentinal layer along the basal edge of the tooth germ.

While it seems that the von Korff fibers, where present, become a part of the dentin, their numbers are too few to account for the full mass. It has been observed that dentin forms in the absence of von Korff's fibers. It must be considered, therefore, that the preponderant part of the collagenous fibrils in the dentin are formed at the junction between the outer papilla cells and the dentin. There seems to be a good possibility that the cell cytoplasm enters into the formation of the fibers.

Von Korff[1, 2] investigated the development of dentin in the pike and found that it proceeded entirely by the apposition of connective tissue fibrils from the pulp. However, he gives only a schematic drawing of the dentin formation. In Fig. 3 of his work,[2] he shows a photomicrograph with the following text: "Tangential section through a tooth germ in pike (Zenker, Mallory). From the dental pulp, numerous bundles of connective tissue fibers pile themselves up against the uncalcified dentinal stratum (growth by apposition)." The section appears to be one of a tooth which is practically fully formed. The fibers all proceed in the longitudinal direction of the tooth from as far basally in the dental papilla as the illustration shows. They extend over into the dentinal layer at various levels. Both the stage of development and the other aspects of the picture correspond fairly exactly to Fig. 151 in Tomes' *Dental Anatomy*,[5] which has the following text: "Hinged tooth in pike. —b. Elastic rods formed of uncalcified trabeculae which might have become bone." Thus, Tomes takes the view that the fibers would have initiated the formation of osteodentin if the tooth had not been hinged, and, therefore, would have nothing to do with the formation of the ordinary dentin. Thus, very little can be deduced from von Korff's work on the formation of dentin in osseous fish.

Weidenreich[6] found that the outer area of the pike tooth is formed of bundles of coarse fibers from the dental papilla, which enter the dentin along the whole border of the dental papilla, and which are orientated longitudinally to the tooth. He gives a photomicrograph which appears to show this formation. It appears that Weidenreich's Fig. 6 is an oblique section of a tooth germ where only the basal part of the tooth has been included in the section, whereas he shows a true longitudinal cut through the basal part of the tooth germ in *Gadus morrhua* (Weidenreich's Fig. 9). The latter corresponds to my findings in *Merluccius merlucius* (Fig. 7) and, if we accept that Weidenreich's section of pike was obliquely cut, then it can be assumed that the orientation of fibers for the pike is similar.

Weidenreich drew attention to the fact that the ends of the coarse fibers which proceed from the dental papilla into the dentin in the pike are easily torn by the knife during the cutting of the sections, and lose their orientation to their original position. The same artifact was often observed in the present material, especially in *Gobius niger* (Fig. 8).

The formation of the dentin in *Gadus morrhua* is described by Weidenreich in a way which, in general, coincides with my findings in *Merluccius merlucius*. Also, in advanced stages of the dentin formation, Weidenreich saw individual fibers which originated from the central area of the dental papilla and entered the dentin. In the present material, such fibers were seldom found at later stages of the dentin formation in *Merluccius merlucius*.

In *Leuciscus rutilus*, Weidenreich found an outer layer of dentin with coarse fibers extending longitudinally, as in *Gadus morrhua*. Inside this there developed a dentin with finer fibers which were not formed from the papilla fibers, except for a few isolated fibers which became embedded in the dentin. Weidenreich considered that the peripheral papilla cells participate in the formation of this inner dentinal layer. He observed the fine-fibered "circumpulpal" dentin developing in areas where typical dentinoblasts were not present, for example, in sharks. Therefore, he accepted that the differentiation of fibrils in the formation of dentin does not proceed by transformation of protoplasm, but that the whole process at most must take its start from the surface of the cell body.[7]

According to my previous investigations of *Squalus acanthias*,[3] the organic foundation of the mesodermal enamel is a product of the mesodermal pulp of the tooth germ. In the formation of mesodermal enamel, no typical dentinoblasts are present, but the cytoplasm from the peripheral papilla cells coalesces in the papilla tip. The fibroglia fibrils of the cytoplasm are transformed into collagenous fibrils. This, along with the transformed cytoplasm, becomes the organic foundation of the mesodermal enamel. This suggests that a hard tooth tissue may come into existence by transformation of cytoplasm without cells being differentiated into typical dentinoblasts.

Weidenreich's assumption of two kinds of dentinal fibrils in osseous fish, each with a different formation, has been confirmed by these researches. However, the separation of these into a coarse-fibered "Mantledentin" and a fine-fibered "circumpulpal dentin" does not seem justified.

SUMMARY

The collagenous fibers of the dentin in osseous fish can be classified into two groupings by their direction and coarseness. First, argyrophil fibers from the dental papilla are so arranged as to form a layer of coarse, longitudinally arranged fibers. In certain species (*Merluccius merlucius*), the whole outer dentinal layer is of this type.

In *Gobius niger*, *Labrus bergylta*, and *Ctenolabrus rupestris*, a second zone of dentin with radially arranged, finer fibers is found between the longitudinally arranged, coarse fibers and the columnar epithelium of the tooth germ. This

forms the outermost layer of the tooth from the mesodermal enamel to the basal edge of the tooth.

The formation of collagenous fibers proceeds so rapidly that a sharp boundary is created between the peripheral papilla cells and the newly formed dentin.

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REFERENCES

1. von Korff, K.: Zur Histogenese der bindegewebigen Stützsubstanzen niederer Wirbeltiere, *Anat. Anz.* (Ergänzungsheft) **37**: 128, 1910.
2. von Korff, K.: Ueber das Wachstum der Dentingrundsubstanz verschiedener Wirbeltiere, *Ztschr. f. mikr.-anat. Forsch.* **22**: 445, 1930.
3. Kvam, T.: The Development of Mesodermal Enamel on Piscine Teeth, Trondheim, 1950, Det Kgl. Norske Videnskabers Selskab.
4. Kvam, T.: On the Development of Dentin in Fish. I. *Squalus acanthias, J. D. Res.* **32**: 280, 1953.
5. Tomes, C.: A Manual of Dental Anatomy, ed. 8, New York, 1923, The Macmillan Company.
6. Weidenreich, F.: Ueber den Bau und die Entwicklung des Zahnbeins in der Reihe der Wirbeltiere, *Ztschr. Anat.* **76**: 218, 1925.
7. Weidenreich, F.: Ibid., p. 241.

THE INTERPRETATION OF THE X-RAY DIFFRACTOGRAMS OBTAINED FROM HUMAN DENTAL ENAMEL

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X-RAY diffraction studies are applied to dental tissues and bone to obtain information about the constituents and their structural arrangement in these tissues. They supplement information obtained by studies with other tools, such as chemical analyses, radioactive tracers, optical studies in plain and polarized light, and histologic studies on mature and developing tissues. Each tool furnishes information with its own particular limitations; together they can give a more complete understanding of the structure of these tissues and of their development. By extending these studies to pathologic deviations in the development of the tissues caused by metabolic disturbances, to changes in structures induced by disease, and to new structures produced by experimental methods, as pulp capping, we hope to help the dentist repair and prevent damages to dental tissues caused by metabolic disorder and disease.

In a previous paper[1] we described a diffraction microcamera for routine work with thin sections of teeth, bone, and other biologic preparations. The camera is a short cylindrical chamber. A lead glass capillary, inserted in the center of the front plate, serves as slit system, defining direction and aperture of the entering x-ray beam. The x-ray then passes through the specimen, which is mounted immediately over the inner end of the capillary. A flat film, 15 mm. distant at the opposite end of the chamber, records the rays diffracted by the specimen, while the undiffracted beam leaves through a center hole in the film and in the back of the camera.

When an x-ray diffractogram (the record of the diffracted rays on the film) is examined, there are two specific aims: (1) to identify the substance or substances causing the diffraction, and (2) to describe the directional arrangement of the diffracting matter in the specimen. The diffraction pattern is typical for the crystalline substance of the specimen, provided certain conditions regarding the arrangement of the diffracting matter in the x-ray beam obtain. However, deviations from these conditions may profoundly alter the appearance of the diffraction pattern. If one wishes to deduct all information possible from such variations in the pattern, utilizing the x-ray diffraction tool to the limits of its powers, one is in need of some crystallographic knowledge.

Although this article deals chiefly with diffraction patterns obtained from dental enamel, much of the argumentation will hold also for dentin and bone, to which we shall occasionally refer.

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A. Powder Pattern.—When a beam of monochromatic x-rays passes through a material which consists of many small crystallites having random orientation, as in a finely-ground powder, the x-ray pattern obtained on the flat film consists of a series of concentric circular rings, Debye-Scherrer rings, and is called a powder pattern (Fig. 1). This pattern is highly characteristic of the diffracting material, and it is widely used for identification of crystalline materials.

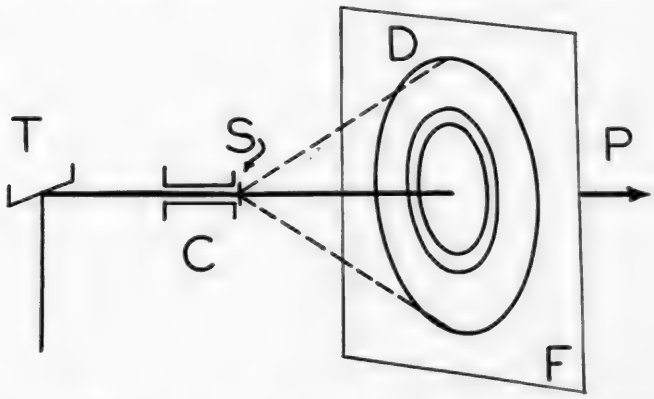


Fig. 1.—Production of an x-ray diffractogram. Characteristic x-radiation emitted by target, *T*, in x-ray tube is collimated by a capillary, *C*, and passes through specimen, *S*. The diffracted rays, *D*, are recorded on film, *F*, while the primary beam, *P*, passes through a center hole in film.

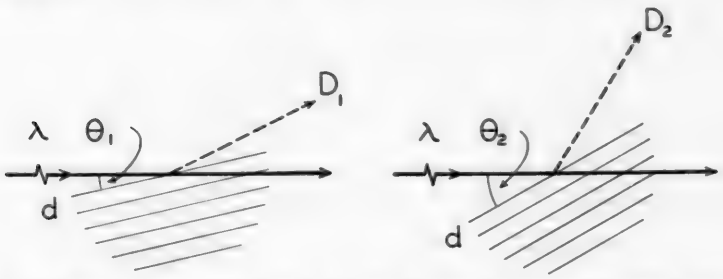


Fig. 2.—Bragg reflection of x-ray by set of atomic planes in crystal occurs when $\sin \Theta = n\lambda/2d$; d = spacing of atomic planes; D_1 = first order reflection ($n = 1$); D_2 = second order reflection ($n = 2$).

From the ring diameter on the film and the specimen film distance, the glancing angle, θ, under which the x-ray is reflected (really diffracted) by a certain set of parallel equispaced atomic planes in the crystallites of the powder is calculated. The spacing, d, of this set of planes is calculated using the Bragg equation, $n\lambda = 2d \sin \theta$, in which λ is the wave length of the x-ray, and n is a whole number, the order of reflection. This equation states the conditions under which a reflection may occur (Fig. 2). For every other ring diameter the corresponding d spacing of the set of planes producing the ring is calculated. The substance may now be identified by consulting a card index[2] in which the powder diffraction patterns (d spacings and relative intensities) for about 4,000

substances have been arranged according to the spacings of their three strongest reflections.

Among the many spacings in a diffraction pattern, according to well-established rules, three certain spacings may be selected as the spacings along the fundamental crystallographic axes of the crystal. Having decided on these crystal axes a, b, and c, and the angles between them, the spacings of all other atomic planes in the crystal can be calculated and checked with the observed list of spacings. The length of the axes and their directions determine the size and shape of the unit cell of the crystal, which by repetition in the three directions builds up the whole crystal.

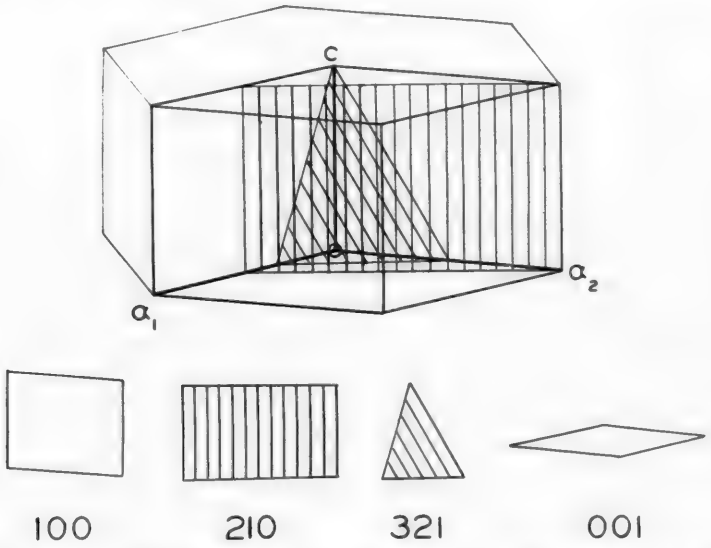


Fig. 3.—Unit cell of hexagonal crystal with orientation of the crystal axes and of some planes. The axis ratio is that of an apatite.

The atomic planes are named by their Miller indices, which are the inverse ratios of the plane's intersections with the crystal axes, and thus define the plane's orientation in the crystal with regard to these axes (Fig. 3). The x-ray reflection from a 210 plane is also named 210. The second order of this is the 420 reflection.

The diffractogram of powdered enamel is pictured in Fig. 4. The continuous rings indicate random orientation of the crystallites in the plane normal to the x-ray beam. From the sequence of the rings the presence of a crystalline material which belongs to the hexagonal system, in which the hexagonal c axis is intersected at right angles by the two equivalent a and b axes with 120 degrees between them, is concluded (Fig. 3). In particular, the diameters of the rings and their intensities so closely match those in the diffractogram of apatite that there is no doubt about this material's belonging to the apatite group.

In the first x-ray diffraction study of enamel, Gross[3] established this identity and gave the approximate dimensions of the unit cell. He also found that enamel has a fiber structure. However, since then discussions on details of the apatite and tooth problems have induced many workers to continue the x-ray investigations, employing new and more refined instruments as they became available in an endeavor to illuminate specific phases, and to answer specific questions on the apatite problem.

The apatites are a crystalline form of calcium phosphates. The theoretical calcium hydroxy apatite contains within one unit cell: 10 Ca^{++}, 6 $(PO_4)^{---}$, 2 $(OH)^-$. The $(OH)^-$ ions can be substituted, e.g., by F^- or Cl^- resulting in a fluoro- or chloro-apatite. Also the Ca^{++} and perhaps the $(PO_4)^{---}$ ions can be substituted to some extent by other ions. Any such substitution is accompanied by smaller or larger changes in the unit cell dimensions, the length of the a axis

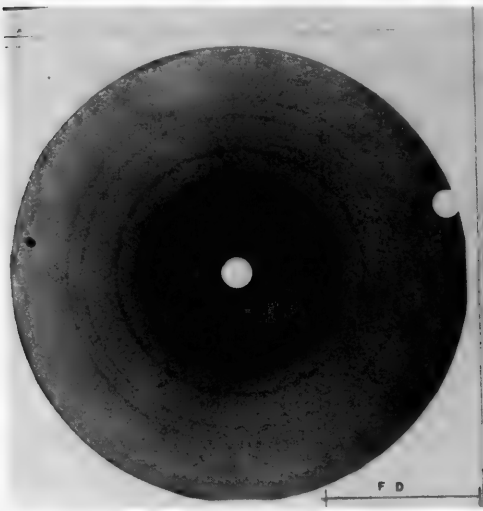


Fig. 4.

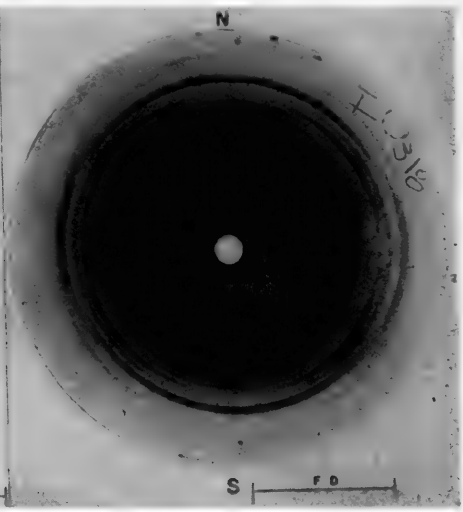


Fig. 5.

Fig. 4.—X-ray diffractogram of powdered dental enamel. The sequence of the diameters and intensities of the rings identifies the crystalline substance of the enamel as an apatite.

Fig. 5.—Diffractogram of thin section of human enamel (sagittal plane of incisor). *N-S*, projection of fiber axis which is perpendicular to x-ray beam. *F D*, film distance from specimen.

usually being more affected than the length of the c axis. This causes a change in the c/a ratio, which easily can be detected on the diffractogram by the greater shift of the (*hko*) reflections relative to the (*ool*) reflections. The substitutions are to be isomorphous. This implies that the result of a, say, 50 per cent substitution of $(OH)^-$ by F^- is not a mixture of one-half (OH) apatite and one-half F apatite, but one homogenous (OH, F) apatite with random distribution of the $(OH)^-$ and F^- ions among the available positions in the crystal, whose unit cell dimensions and other physical properties are nearly halfway between those of pure (OH) apatite and pure F apatite. Thus, the unit cell dimensions can be utilized as a clue to an apatite's composition.

B. Fiber Pattern.—From a thin slab of enamel a diffraction pattern as shown in Fig. 5 is obtained. The enamel rods lie in the plane of the slab approximately parallel to the *N-S* direction on the film. The rings of the powder pattern have split into segments, or arcs, indicating that the enamel consists of many small crystallites, perhaps $\frac{1}{50}$ to $\frac{1}{500}$ of the thickness of an enamel rod. These crystallites are not randomly arranged, but their c axes prefer an orientation more or less parallel to a certain direction which is called the fiber axis of the crystal aggregate. In general, the better the parallel orientation, the shorter will be the arcs on the film.

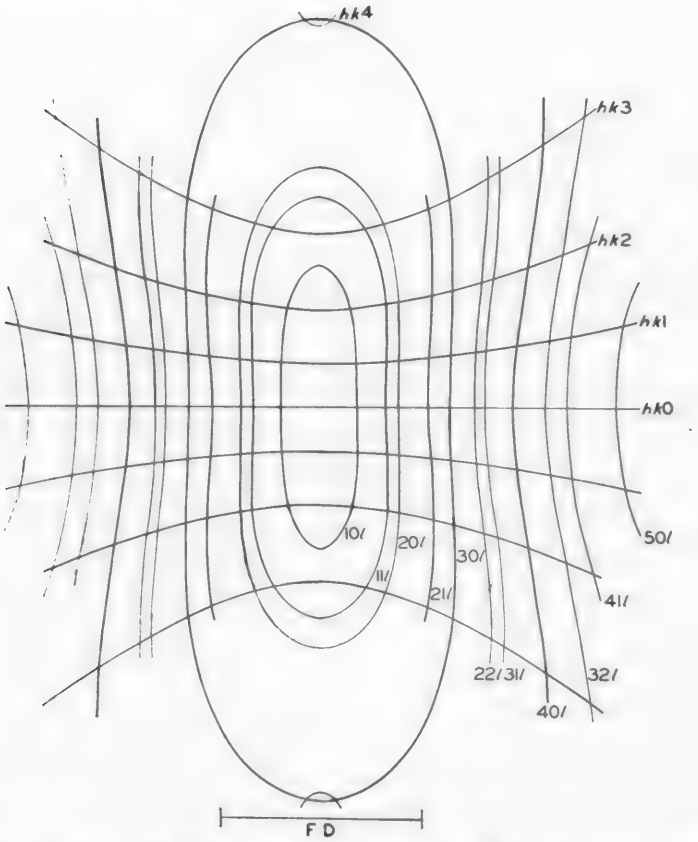


Fig. 6.—Diagram of the diffraction pattern on flat film obtained when a single crystal of apatite is rotated about its c axis which is perpendicular to the x-ray beam. The reflection spots are located at the intersections of the horizontal and vertical lines.

The projection of the fiber axis, *N-S* on Fig. 5, passes through the centers of the (*ool*) reflections, e.g. 002 and 004. Perpendicular to this "meridian" lies the "equator" which passes through the (*hko*) reflections, e.g. 210 and 300, etc. The centers of the other arcs are arranged as the reflections in a rotation

pattern of a single crystal of apatite which, during the exposure, is rotated about its c axis. When this rotation axis is set perpendicular to the x-ray beam, the reflections lie on hyperbolic layer lines, for each of which l remains constant, and on the row lines for which h and k are constant.

Fig. 6 is a chart showing these layer and row lines. Their intersections are the spots where reflections can possibly appear. Besides, the reflections must lie on their Debye-Scherrer rings. This chart has been calculated for

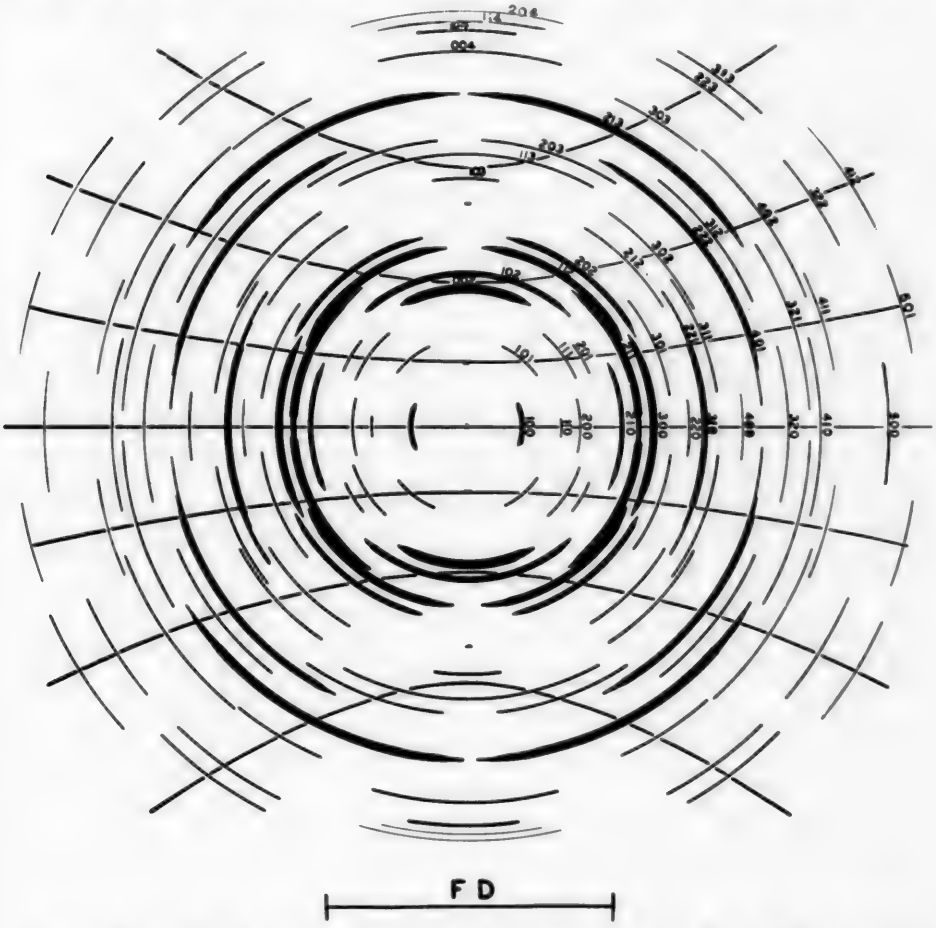


Fig. 7.—Diagram of the diffraction pattern of human enamel on flat film (same orientation as Fig. 5). The reflections are indexed and their intensities are indicated by thickness and length of the lines.

human enamel apatite with the aid of Bernal's equations.[4] It has been extended beyond Bernal's Chart II to include the outer reflections appearing on our films. The chart can be reduced, or the diffractograms can be enlarged, to equal film distance $F\,D$; thus, indexing of the reflections in the diffractograms is made easy.

Fig. 7 is a diagram of the reflections of enamel (fiber axis perpendicular to x-ray beam) as they appear on the flat film, with the layer lines of Fig. 6 superimposed. The reflections near the meridian, namely 002, 004, 103, 104, 114, 204, and 214, do not fall on their layer lines. They, and also the extensions of all other reflections below and above their layer lines on the chart, have been diffracted by crystallites whose c axes are inclined forward or backward, respectively, from the normal to the x-ray beam; they are more or less declining away from the fiber axis. The intensity distribution along the 002 arc gives a rough idea of the directions of the c axes of the crystallites. In a well-oriented section of human enamel most of the crystallites have their c axes deviate less than

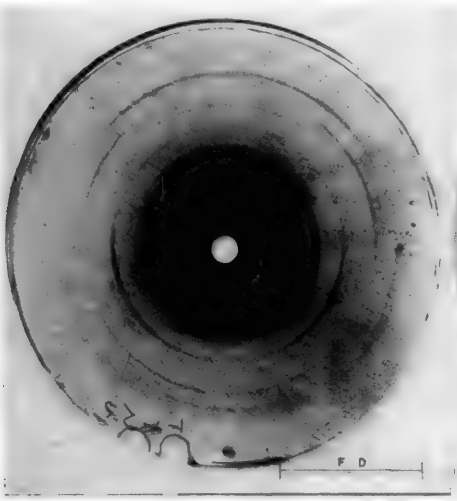


Fig. 8.

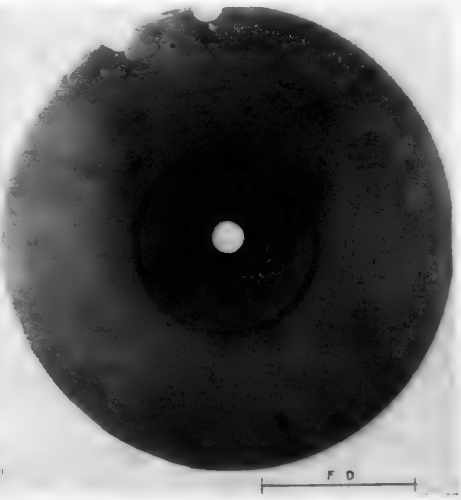


Fig. 9.

Fig. 8.—Diffractogram of thin section of human dental enamel, fiber axis tilted approximately 45 degrees from perpendicular to x-ray beam, upper end toward x-ray tube. Tilting destroys the symmetry across the equator.

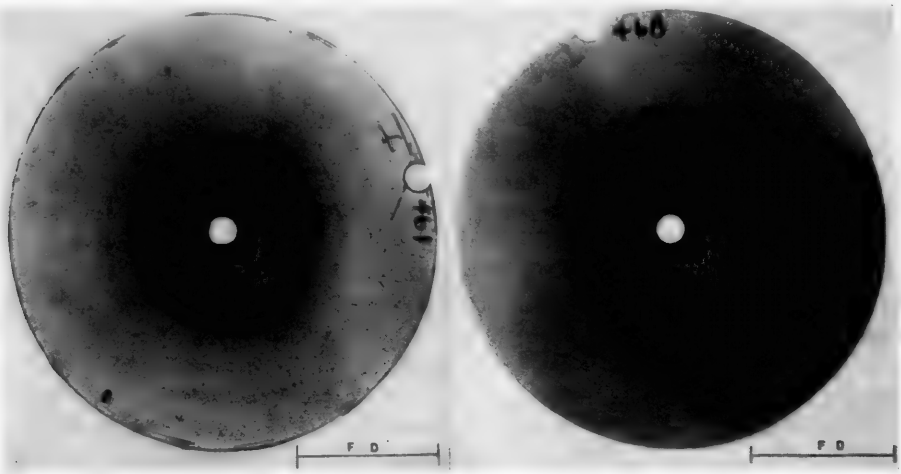
Fig. 9.—Diffractogram of thin section of human dental enamel, fiber axis nearly parallel to x-ray beam. The equatorial reflections are full circles, demonstrating the rotational symmetry about the fiber axis. The meridional reflections (Fig. 5) are highly weakened. Note almost complete absence of 002 and 102. The shadow of the specimen has blotted out the reflections in the lower half of this diffractogram.

20 degrees from the fiber axis, and very few deviate more than 35 degrees. There are, however, other enamel sections which show much less preferred orientation. Human dentin always gives a pattern with complete 002 rings, usually of constant intensity all the way around; occasionally, however, two opposite sides of the 002 ring are slightly intensified due to a slight preferred orientation of the c axes of the crystallites.

The crystallite arrangement is that found within the enamel rod. We have examined bundles of few enamel rods, and have always obtained smooth arcs on rings and never observed spots or spotty arcs suggesting the presence of individual crystallites of appreciably larger size. Similar observations have been previously described,[5, 6] but it seemed logical to repeat these experiments with samples 1,000 or more times smaller than previously had been used.

When the fiber axis is tilted, upper end toward the x-ray tube, the reflection arcs shift along their Debye-Scherrer circles down toward S, and the pattern symmetry above and below the equator is lost. Fig. 8 shows the pattern when the fiber axis is tilted approximately 45 degrees from the perpendicular toward the x-ray tube. From the shift of the reflections it is possible to determine the tilt angle of the fiber axis. If the fiber axis is tilted so far that it becomes parallel to the x-ray beam (tilt angle 90 degrees), the arcs of some reflections, i.e. those with zero or low l index, have closed to form rings which show that there is an approximately rotational symmetry about the fiber axis (Fig. 9). Other reflections, i.e. those with high l indices, have disappeared.

Fig. 9 also demonstrates another important point. The complete rings are evidence of random orientation, but only in the projection upon the plane normal to the x-ray beam; the diffractograms (Figs. 5 and 8) taken in other directions reveal the true preferred orientation of the crystallites in the same



A. *B.*

Fig. 10.—Diffractogram of bone (spicule from neck of goose) showing a considerable degree of preferred orientation. *A*, Fiber axis perpendicular to x-ray beam; *B*, fiber axis parallel to x-ray beam.

sample. The difference between this diffractogram (Fig. 9) and that of complete random orientation (Fig. 4) becomes apparent if the intensities of the rings are compared. Preferred orientation is likely to influence severely the relative intensities of the reflections, e.g. the 002 reflection in Fig. 4 is stronger than the 210 reflection, while in Fig. 9 it is hardly visible, or even absent. Any intermediate intensity may be expected, depending upon the degree of preferred orientation and on the orientation of the fiber axis relative to the x-ray beam.

Neither dentin, as mentioned previously, nor bone exhibit that high degree of preferred orientation. In fact, most published reports[6, 7] emphasize the random orientation of their crystallites. However, Thewlis[6] has observed dentin with some degree of preferred orientation, and we have several diffractograms

on which a slight orientation is indicated, but not to such a degree that the intensity of the 002 reflection at the equator of the diffractogram has been weakened to the intensity of the fused 210 and 102 lines. In some bone spicules (e.g. from the neck of a goose) we observed a considerably higher degree of orientation (Fig. 10, *A*). If such samples are x-rayed parallel to the fiber axis (Fig. 10, *B*), the 002 reflection, particularly, will suffer a reduction in intensity. Now, it is the intensity variation, particularly of this 002 reflection, which has at times been used to demonstrate the existence of different types of dentin,[7] or the variation was thought to be due to differences in the chemical composition of the apatite, or to the particular shape of the apatite crystals in the dentin.[7] In this case it could be argued that the weakened, though broadened, 002 line indicates platelet shape normal to the c axis, rather than needles parallel to the c axis, although other experimental evidence does not support this conclusion. It is obvious that, first the effect of a possible preferred orientation in the specimen must be considered, if a thin section is used, or must be excluded by the use of a powdered sample, before one attempts to employ any observed intensity variations for other deductions.

As far as we now know, no such variations occur in the composition of the mineral matter of enamel and bone or such substitutions in its apatite which would cause visible changes in the intensity ratio of the x-ray lines on the films.

C. Crystallite Size.—The diffractogram of enamel exhibits fairly sharp lines. However, they are not as sharp as those obtained with the powder of a well-crystallized natural apatite. Dentin and bone, on the other hand, give lines which are more diffuse (Fig. 10), so much so that the 211 and 112 reflections are not resolved, and even the 300 reflection is fused with them to form one broad band.

The diffuseness of the lines is due to limitation of the crystal size. Since these lines are a diffraction effect of a grating (atomic planes in the crystal), the limitation of the number of equidistant diffraction centers (atomic planes) will cause weaker and broader (more diffuse) lines with less contrast against the background of the general scattered radiation on the film. From such line broadening one tries to derive values for the crystallite size. That width of the line is usually measured where the intensity is half the peak intensity. The relationship is most useful when the size of the crystallites fall within the range between 1,000 and 100 Å. If the crystallites are larger, the broadening effect is very small, and if the crystallites are smaller the lines are too diffuse to permit sufficiently precise measurement of the intensity differences between line and background.

From the profiles of photographically recorded diffraction lines of powdered materials, Jensen and Moeller[8] calculated a crystallite size of 200 Å for dentin and up to 600 Å for human enamel. Certain inconsistencies still remained. In particular, the crystallite size calculated from the higher orders of reflection was smaller than that calculated from the low orders. Such an effect would be expected, if the line broadening is not only due to limitation of the crystallite size but also due to lattice distortions in the crystallites. It may be easily con-

ceived that occlusion within the apatite crystallites of finely-dispersed foreign matter produces a distortion of the lattice.

More precise line profiles than with photographic methods can be plotted with the Geiger counter spectrometer. From the profile of the 002 reflection of a mixed sample of human incisor enamel* we calculated an average crystallite size of 870 Å along the c axis. From the 004 reflection we also obtained a lower value, 480 Å, and the 410 reflection gave 550 Å. The 002 reflection of powdered dentin gave 290 Å. These values were obtained in an evaluation of the line profiles according to the method of Alexander, who has made a detailed analysis of the instrumental line-broadening effects in the new Norelco spectrometer.[9] Smaller sizes are calculated with Jones's[10] method which, however, had been designed for work with powder cameras where the instrumental broadening effects are different from those in the spectrometer. Jensen[11] used an equation described by Warren and Biscoe[12] which is based on simplifying assumptions whose deviations from actual conditions cannot be easily checked. A more stringent treatment of the experimental data with Fourier series analysis will be published at a later date. Measurements with the Geiger counter spectrometer require comparatively large samples of powdered material (0.15 Gm.), so that for a study of the local variations of the crystallite size, as well as of any other factor, one still will have to resort to photographic methods.

D. Proper and Spurious Reflections.—The diffractograms of normal human enamel are those of an apatite. The lines are not split into doublets, from which fact we conclude the presence of only one apatite, and not of a mixture of two apatites with different axis lengths. The c/a axis ratio[13] is close to that of precipitated hydroxy apatite. All reflections which we have observed fit into this apatite pattern and we have observed on the diffractograms all thirty-five apatite reflections which are possible up to 004 with the exception of the five weakest ones: 220, 302, 400, 401, and 411. The (hkl) and ($\bar{n}kl$) which have identical spacings but different intensities, of course, cannot be resolved from each other in enamel. Near the center of our diffractograms (Fig. 5) appear some reflections of planes with large spacings which previously were not listed in the diffractograms of enamel. They were identified as the 100, 110, and 101 reflections of apatite. The 001 and 003 reflections are absent, as is required by the crystallographic space group of apatite. Thus, if different ions take positions along the c axis, they will be not in an ordered, but in a random, sequence.

The powers of this technic for revealing the weak reflections come from the low background scatter in the microcamera and from the use of oriented thin sections of enamel. Here the diffracted energy is concentrated in more or less short arcs, instead of being distributed over the whole circle as is the case in the powder technic. Thus, a greater contrast against the background is developed. Furthermore, individual reflections are separated along a circle, even if their spacings are almost the same, e.g. 211 and 112.

*The profile is shown in Trautz, O. R.: The Use of X-Ray Diffraction in Dental Research, Ann. Dent. 12: June, 1953. Recently the name of the instrument has been changed from spectrometer to diffractometer.

The composition of enamel does not correspond exactly to the hydroxy apatite formula. The phosphate may be accompanied by about 7 equivalents per cent carbonate and 1 equivalent per cent citrate, the calcium by 2 equivalents per cent magnesium and 0.6 equivalent per cent sodium. However, scrutinizing more than 250 diffractograms, we found no indication whatsoever of any other crystalline components' being present in enamel besides the one apatite, in spite of the fact that we had the most favorable conditions for their detection, since between the apatite arcs there is ample space left for other reflections to be visible. The previously mentioned accessory components, therefore, must be contained in the enamel, either as isomorphous substituents in the apatite or as so finely dispersed (amorphous) matter that they cannot produce the diffraction lines which are characteristic of their crystallized form.

Sometimes on strongly exposed diffractograms, one or two spurious weak lines are discovered inside the strongest reflections 002 and 300. These are easily identified as the Kβ reflection on 002 or 300, since the position of a reflection on a film (i.e. its sin θ value) varies proportionally with the wave length of the radiation. They could be completely filtered out by the use of a thicker nickel filter.

Another effect of incompletely monochromatized radiation may be noted: a broad, diffuse ring near the center. Reference to it is made at various places in the literature,[7, 14, 15] and different explanations for it have been advanced. However, the diffuse reflection is a part of the continuous spectrum reflected by the strongly reflecting 002 planes, or by the strongly reflecting 112-211-300 triplet, as becomes evident by the following calculation. Inserting into the Bragg equation the known spacings of 002 or the triplet and the glancing angle θ of the inner edge of the diffuse reflection, we obtain a wave length which is equal to the short wave length limit of the continuous spectrum. This depends on the peak voltage across the x-ray tube, according to λ minimum $=$ 12.34 per KVP. For 40 and 35 KVP we find λ (min.) $=$ 0.31 and 0.35 Å, respectively. For the outer edge of these diffuse reflections we find a wave length of 0.40 Å, which is the K absorption edge of the silver contained in the photographic emulsion. Radiation of a shorter wave length than this is more strongly absorbed in the emulsion, and thus has a stronger photographic action than longer wave lengths.

These diffuse "white" reflections, when they occur, are always some distance inside very strong characteristic (Kα) reflections. They become particularly noticeable with comparatively thick specimens, in which the softer characteristic radiation is absorbed to a large extent, and with molybdenum radiation, whose characteristic radiation is accompanied by a comparatively higher hump of the continuous spectrum than is the case with copper radiation.

SUMMARY

Some of the x-ray diffraction methods used in studies of the structure of dental tissues have been presented. A logical approach to the interpretation of their diffractograms is offered.

REFERENCES

1. Klein, E., Trautz, O. R., Addelston, H. K., and Fankuchen, I.: An X-Ray Diffraction Microcamera and the Specimen Preparation for the Study of Tooth Structure, *J. D. Res.* **30**: 439, 1951.
2. X-Ray Diffraction Data Cards, American Society for Testing Materials, Philadelphia, 1950.
3. Gross, R.: Die kristalline Struktur von Dentin und Zahnschmelz, Berlin, 1926, Festschr. Zahnaerztl. Inst. Univ. Greifswald, p. 59.
4. Bernal, J. D.: Interpretation of X-Ray, Single Crystal, Rotation Photographs, *Proc. Roy. Soc., London;* **A 113**: 130, 1926.
5. Bale, W. F.: A Comparative Roentgen-Ray Diffraction Study of Several Natural Apatites and the Apatite-Like Constituent of Bone and Tooth Substance, *Am. J. Roentgenol.* **43**: 736, 1940.
6. Thewlis, J.: The Structure of Teeth as Shown by X-Ray Examination, London, 1940, Medical Research Council, Spec. Report Series 238.
7. Bale, W. F., Hodge, H. C., and Warren, S. L.: Roentgen-Ray Diffraction Studies of Enamel and Dentine, *Am. J. Roentgenol.* **32**: 369, 1934.
8. Jensen, A. T., and Moeller, A.: Determination of Size and Shape of the Apatite Particles in Different Dental Enamels and in Dentin by the X-Ray Method, *J. D. Res.* **27**: 524, 1948.
9. Alexander, L. E.: Personal Communication, also *J. Appl. Physics.* **21**: 137, 1950.
10. Jones, F. M.: The Measurement of Particle Size by the X-Ray Method, *Proc. Roy. Soc., London* **A 166**: 16, 1938.
11. Jensen, A. T.: Determination of Particle Size by the X-Ray Powder Method, Det kgl. danske Videnskabernes Selskab, Mat.-Fys. Medd. **20**: No. 8, 1943.
12. Warren, B. E., and Biscoe, J.: The Structure of Silica Glass by X-Ray Diffraction Studies, *J. Amer. Ceramic Soc.* **21**: 49, 1938.
13. Trautz, O. R., Klein, E., and Addelston, H. K.: Variations in the X-Ray Diffractograms of Dental Enamel of Man and Shark, *J. D. Res.* **31**: 472, 1952.
14. Clark, G. L., Bucher, C. S., and Lorenz, O.: An Extension of X-Ray Researches on the Fine Structures of Colloids to Normal and Physiological Human Tissues, *Radiology* **17**: 482, 1931.
15. Henschen, C., Straumann, R., and Bucher, R.: Ergebnisse roentgenspektrographischer Untersuchungen an Knochen. Kristallitbau des anorganischen und des organischen Knochens, *Deutsche Ztschr. f. Chir.* **236**: 485, 1932.

A SIMPLE TECHNIC FOR THE HISTOLOGIC PREPARATION OF THE ORGANIC MATRIX OF TOOTH ENAMEL

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ADVANCEMENT in the histopathology of dental caries appears dependent on improved histologic procedures for recovering the organic enamel matrix in decalcified sections. Sognnaes,[1] in a review of the difficulties involved, pointed out that an essential factor is immobilization, or physical support, of this delicate structure during the decalcification process. To maintain the true arrangement of the matrix of the enamel rods, a supporting framework is needed. A method recently devised by us satisfies this requisite and has given consistently good results with the additional advantage of requiring no elaborate apparatus. The specimen is supported externally by an agar gel which is permeable to decalcifying and embedding substances. Additional support is obtained by use of a decalcifying fluid of high viscosity.

Following extraction, the whole tooth is fixed in 10 per cent neutral formol. The anatomic crown is then separated from the root and cut into quarters with a revolving vulcarbo disk. All dentin is ground away from the enamel with a diamond stone. It is essential that all of the dentin be removed, because the relatively long period required for its decalcification results in a partial or complete decomposition of the lacelike enamel matrix. To determine whether dentin remains, the specimen is placed in 10 per cent neutral formol to which aqueous light green has been added (10 drops of 1 per cent aqueous light green in 50 c.c. of 10 per cent neutral formol) for a twenty-four hour period. The specimen is then examined under 7X magnification for dentin fragments which, in contrast to the enamel, stain more intensely. The enamel is then placed in a small dish and covered with a 2 per cent aqueous solution of agar (Difco Laboratories, bacteriological) melted and cooled to about 60° C. Previous filtration of the agar while still very hot gives it greater transparency and facilitates better orientation of the specimen on sectioning. Upon solidification of the agar gel, a small block just encompassing the enamel is cut from it. The block is then dehydrated in increasing concentrations of alcohol and in alcohol-ether, as is usual for celloidin embedding, and afterward placed for several days in 5 per cent nitrocellulose.

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At this stage, the specimen is decalcified with a modification of the acid-celloidin method of Bodecker.[2] To 45 ml. of 10 per cent nitrocellulose, 5 ml. concentrated nitric acid is cautiously added, drop by drop and with constant agitation. The progress of decalcification in this solution can advantageously be followed by roentgenograms, as suggested by Miles.[3] Dependent upon the size of the specimen of enamel, the time required for decalcification has been

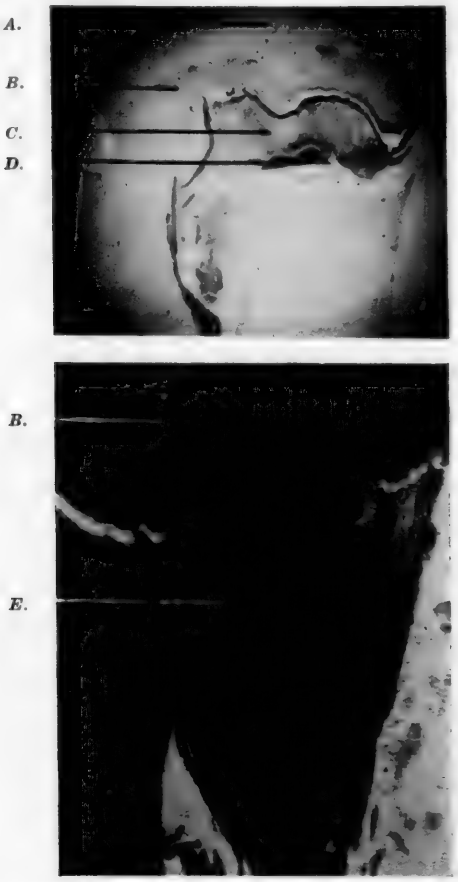

Fig. 1.—Adult human tooth embedded in agar and decalcified in acid celloidin. Aniline blue, 6 μ, ×25, ×250. *A*, Celloidin; *B*, agar; *C*, full thickness of enamel matrix; *D*, dentin; *E*, enamel rods in longitudinal section.

found to vary from twelve to seventy-two hours with this method. Extreme care in handling the specimen, with as little agitation as possible, is now essential. At the end point of decalcification, the excess acid is removed with three changes in 50 ml. quantities of 10 per cent nitrocellulose.

Infiltration of the specimen is continued by leaving the specimen in a fourth change of 10 per cent nitrocellulose for one week, 25 per cent nitrocellulose one week, and 50 per cent nitrocellulose for several days. Final hardening

is done by slow air-drying in a closed Stender dish. Sections, cut at 6 μ, are preferably stained loose as the agar does not adhere well to albuminized slides. Although a wide variety, including bacteriologic stains, may be used, aniline blue has been found to be a particularly satisfactory stain for studying normal structure.

The technic is useful for the histologic examination of both normal and carious enamel. Not only is enamel-rod detail well preserved, but the dental plaque is maintained in its true relation to the enamel surface. Conceivably, the method may also have application to other highly calcified structures.

REFERENCES

1. Sognnaes, R. F.: Organic Elements of the Enamel. I. A Study of the Principal Factors Involved in the Histological Preservation of the Organic Elements of Enamel and Other Highly Calcified Structures, *J. D. Res.* **27**: 609, 1948.
2. Bodecker, C. F.: Enamel of the Teeth Decalcified by the Celloidin Decalcifying Method and Examined With Ultra Violet Light, *D. Review* **20**: 317, 1906.
3. Miles, A. E. W.: A Test for the End-Point of Decalcification of Histological Specimens, *Brit. D. J.* **86**: 297, 1949.

STUDIES ON EXCRETION OF ANTIBIOTICS IN HUMAN SALIVA

III. Aureomycin

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EXCRETION of penicillin, streptomycin, and chloramphenicol in the saliva,* a phenomenon of significance in the prophylaxis of bacteremia caused by tooth extraction, has been the subject of previous investigations by us.[1, 2] To date, only one similar study has been reported on aureomycin; it concerns excretion of aureomycin in the saliva following per oral administration,[3] in which measurements of concentration were done at only three time intervals. The purpose of the following study is to measure and compare the concentration of aureomycin in the blood serum and in the saliva at various intervals following intravenous administration.

PROCEDURES

Adult patients who had not been on antibiotic therapy for at least ten days were selected from the ward of the medical service. Another group of subjects chosen from laboratory personnel were used as controls; antibiotic was not administered to them. A single dose of 100 mg. of aureomycin hydrochloride with sodium glycinate buffer (lot No. 6275) was administered intravenously to each member of the experimental group.

Samples of blood and saliva, approximately 5 ml. each, were collected at 0.25, 0.5, 1, 2, 6, 8, 10, 16, and 24 hour intervals after administration of aureomycin. Blood and saliva specimens were collected, stored, and prepared for assay according to procedures previously described.[1] Blood serum and saliva specimens were assayed for aureomycin by a turbidimetric method based on procedures previously described.[4, 5] This method involves the use of standard curves. Table I shows the concentrations of aureomycin in physiologic saline in the standard tubes used in constructing the curves. Unlike the assay for chloramphenicol, the assay for aureomycin did not require a correction for the nonspecific inhibitory action of some salivas on the growth of the test organism. (The range of assay for aureomycin is such that salivary inhibition effects expressible in Klett units would be negligible expressed in equivalent effects of aureomycin.)

RESULTS

Blood Serum.—After a single intravenous administration of 100 mg. of aureomycin hydrochloride, the average concentration of aureomycin in the

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*Saliva as used here should really be designated "spit." Saliva is a digestive fluid secreted by salivary glands, whereas spit includes mucous, as well as saliva secretions.

TABLE I

SOLUTION IN STANDARD TUBES

μG PER ML. AUREOMYCIN IN PHYSIOLOGIC SALT SOLUTION	
SERUM ASSAY	SALIVA ASSAY
1.0	0.500
0.5	0.250
0.25	0.125
0.125	0.063
	0.031

blood serum, in micrograms per milliliter, rose to 2.15 at the end of fifteen minutes, fell to 1.7 at the end of a half-hour, and apparently stayed near that level until the end of the hour when the concentration was 1.88 (average of fifteen values ranging from 1.00 to 3.70). Between the first and second hour

TABLE IIA

CONCOMITANT LEVELS OF AUREOMYCIN IN SERUM AND SALIVA FOLLOWING A SINGLE 100 MG. INTRAVENOUS DOSE OF AUREOMYCIN-HCl WITH Na GLYCINATE BUFFER (LOT NO. 6275)

PATIENT NUMBER	CONCENTRATION OF AUREOMYCIN IN μG PER ML.			
	15 MINUTES		30 MINUTES	
	SERUM	SALIVA	SERUM	SALIVA
1	2.60	0.015	2.2	0.020
2	2.60	0.023	2.0	0.049
3	1.75	0.015	1.5	0.063
4	2.80	0.020	2.0	0.023
5	1.00	0.030	0.8	0.031
Range	2.8	0.030	2.2	0.063
	1.0	0.015	0.8	0.020
Mean	2.15	0.021	1.7	0.037

TABLE IIB

CONCOMITANT LEVELS OF AUREOMYCIN IN SERUM AND SALIVA FOLLOWING A SINGLE 100 MG. INTRAVENOUS DOSE OF AUREOMYCIN HCl WITH Na GLYCINATE BUFFER (LOT NO. 6275)

PATIENT NUMBER	CONCENTRATION OF AUREOMYCIN IN μG PER ML.											
	1 HOUR		2 HOUR		4 HOUR		6 HOUR	8 HOUR		10 HOUR	16 HOUR	24 HOUR
	SERUM	SALIVA	SERUM	SALIVA	SERUM	SALIVA	SALIVA	SERUM	SALIVA	SALIVA	SALIVA	SALIVA
6	1.92	0.026	1.0	0.096	0.58	0.100	–	–	–	–	–	–
7	1.00	0.040	0.85	0.096	0.70	0.100	–	–	–	–	–	–
8	3.70	0.040	1.00	0.026	0.83	0.086	–	–	–	–	–	–
9	2.10	0.030	0.85	0.030	1.00	0.100	–	–	–	–	–	–
10	2.50	0.026	1.5	0.028	0.70	0.150	–	–	–	–	–	–
11	1.28	0.140	0.5	0.055	0.36	0.100	0.075	–	0.048	0.048	–	–
12	1.16	0.350	–	0.140	0.38	0.170	0.150	0.22	0.140	0.140	–	–
13	1.04	0.150	0.46	0.086	0.42	0.125	0.100	0.22	0.086	0.085	–	–
14	1.52	0.125	0.56	0.086	0.48	0.047	0.047	0.25	0.047	0.031	–	–
15	1.75	0.170	0.64	0.086	0.40	0.125	0.125	0.26	0.086	0.086	–	–
16	2.0	0.083	1.28	0.066	0.88	0.078	–	–	0.074	0.035	0	0
17	2.40	0.125	1.36	0.056	0.56	–	0.050	–	0.050	0.031	0.031	0
18	1.12	0.150	1.12	0.063	0.25	0.083	0.039	–	0.039	0.031	0.031	0
19	3.06	0.115	1.28	0.063	0.54	0.115	0.070	–	0.044	0.044	0.031	0
20	1.68	0.170	1.00	0.063	0.50	0.10	–	–	–	–	–	–
Range	3.70	0.350	1.36	0.140	1.00	0.170	0.150	0.26	0.140	0.140	0.031	
	1.00	0.026	0.46	0.026	0.25	0.047	0.039	0.22	0.039	0.031	0.000	
Mean	1.88	0.116	0.95	0.069	0.57	0.106	0.082	0.23	0.068	0.059	0.023	0

the aureomycin concentration fell to 0.9 (a 50 per cent drop); by the end of the eighth hour it was down to 0.23. See Fig. 1 and Tables IIA and IIB.

Saliva.—After the intravenous administration of aureomycin hydrochloride, the average concentration of aureomycin in the saliva, in micrograms per milliliter, rose to 0.0146 in fifteen minutes, continued rising to 0.0372 at the end of one-half hour, and to a peak concentration at the end of one hour of 0.116 (average of fifteen values ranging from 0.026 to 0.350). At the end of two hours it had fallen to 0.069 (a 40 per cent drop). The concentration of aureomycin at the end of the fourth hour indicated a secondary rise to 0.106, almost up to the peak concentration attained at the end of the first hour. From that time on, there was a slow continuous drop until the sixteenth hour. No aureomycin could be detected at the end of twenty-four hours. See Fig. 2 and Tables IIA and IIB.

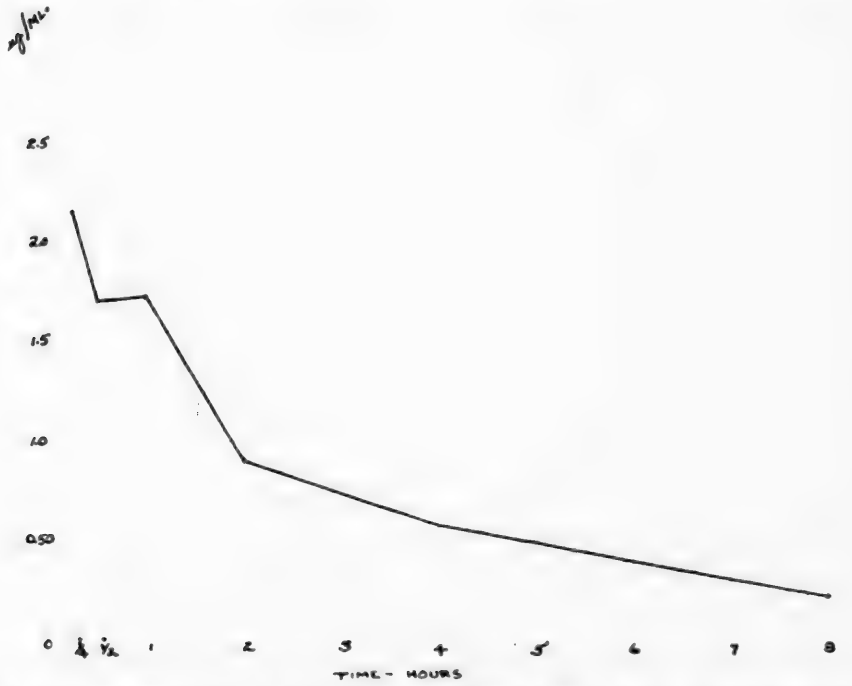

Fig. 1.—Aureomycin. Average serum levels after sodium buffer 100 mg. intravenous dose of aureomycin hydrochloride with glycinate.

Other investigators have not reported a secondary rise in aureomycin excretion in saliva. However, the present study is the only one in which the aureomycin was administered intravenously. Possibly the rapidity with which the antibiotic can enter the saliva through the intravenous route has something to do with this phenomenon; on the other hand, it may be that the secondary rise observed was simply a variation within the range of experimental error.

SUMMARY AND CONCLUSIONS

1. Aureomycin was administered intravenously to a group of patients, and the concomitant levels of aureomycin in the blood serum and in the saliva were measured at various intervals after administration.

2. Intravenous injection of aureomycin is followed by excretion of aureomycin in the saliva within fifteen minutes.

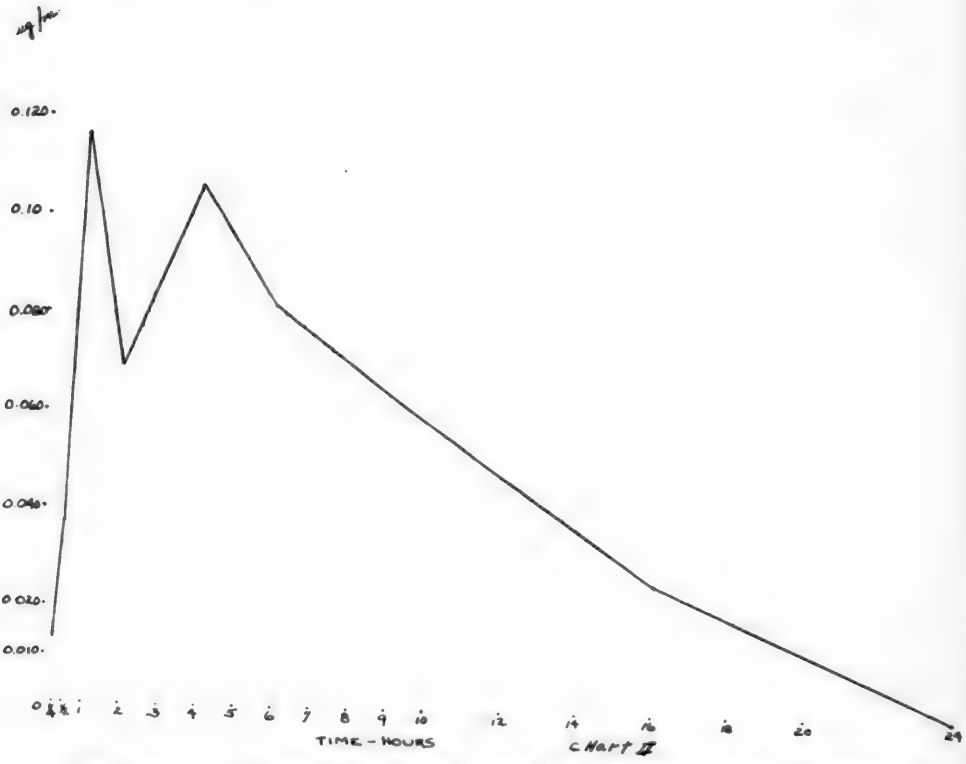

Fig. 2.—Aureomycin. Average saliva levels after 100 mg. intravenous dose of aureomycin hydrochloride with sodium buffer glycinate.

3. The highest concentration of aureomycin in the saliva is observed at the end of the first hour after administration; by the end of the second hour there has been a 40 per cent drop in aureomycin concentration.

4. At the end of the fourth hour there seems to be a secondary rise in saliva to a concentration almost equal to the peak attained at the end of the first hour; this occurs even though the concentration in the blood serum during this interval is falling sharply. Possibly the secondary rise is merely an accidental variation within the range of experimental error, or a true function.

5. After the fourth hour there is a gradual drop in concentration of aureomycin; at the end of the sixteenth hour measurable amounts are still present; at the end of twenty-four hours no aureomycin can be detected.

6. Aureomycin, although given intravenously, manifests a peak level in saliva at the end of one hour. In contrast to chloramphenicol, also given intravenously, the peak level is reached in one-half hour.

REFERENCES

1. Bender, I. B., Pressman, R. S., and Tashman, S. G.: Studies on Excretion of Antibiotics in Human Saliva: I. Penicillin and Streptomycin, *J. A. D. A.* **46**: 164, 1953.
2. Bender, I. B., Pressman, R. S., and Tashman, S. G.: Studies on Excretion of Antibiotics in Human Saliva: II. Chloramphenicol, *J. D. Res.* **32**: 287, 1953.
3. Kraus, F. W.: Antibiotic Levels in Human Saliva, *J. D. Res.* **30**: 495, 1951.
4. Whitlock, C. M., Jr., Hunt, A. D., Jr., and Tashman, S. G.: A Simplified Turbidimetric Method of Aureomycin Assay for Capillary Blood and Other Body Fluids, *J. Lab. & Clin. Med.* **34**: 1682, 1949.
5. Whitlock, C. M., Jr., Hunt, A. D., Jr., and Tashman, S. G.: A Simple Turbidimetric Method for the Assay of Aureomycin, Chloramphenicol, Penicillin, Streptomycin and Terramycin in Capillary Blood and Other Body Fluids, *J. Lab. & Clin. Med.* **37**: 155, 1951.

THE RELATION OF CARIES ACTIVITY TO LACTOBACILLUS COUNTS AND TYPES AND TO THE FLUORIDE CONTENT OF DRINKING WATER

II. Results of Examinations Made After a One-Year Interval

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RECENTLY we have reported evidence that the number of decayed surfaces and the percentage of those having decayed teeth are greater in children in whose saliva a rhamnose-fermenting lactobacillus is the predominating type, than in those in which another type predominates. Children between the ages of 11 to 14 years, from an area having 1 ppm fluoride (Denver), and an area having no fluoride in the drinking water (Boulder), were examined. Lactobacillus counts and types, isolated from saliva taken shortly after the dental examinations, were studied in relation to the extent of decay.

One year after these studies were made the same children were examined again. Lactobacillus counts were made and types were determined in part of the group. It is the purpose of this report to show the relation of the counts and types determined originally to the new decay observed after one year's interval. The changes in the numbers and kinds of lactobacilli in the saliva are also reported.

EXPERIMENTAL METHODS

One hundred fifty-nine of the 197 children originally examined in the Denver area, and 153 of the 188 children in the Boulder area, were re-examined for new decay by one of us (R.A.D.). The saliva from 73 of the Denver children and 70 of the Boulder children was examined for counts and types of lactobacilli. The methods of collecting the saliva and isolating and counting the bacteria were the same as outlined previously.[1] Lactobacilli were typed according to the scheme shown in Table I.

RESULTS AND DISCUSSION

New Caries in the Previously Caries-Free.—Table II shows the percentage of those children with caries at the second examination who were caries-free at the first examination. In the Denver group (1 ppm fluoride in water), 62 per cent of those with high counts and rhamnose-fermenting lactobacilli predominating in their saliva one year previously now showed decay. Forty-eight per cent of those with rhamnose fermenters (both high and low counts) showed decay,

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while only 30 per cent of those with rhamnose-negative lactobacilli were carious. This indicates that children in an area with 1 ppm fluoride in the water, who have no caries, are more likely to develop caries within the next year if their saliva contains large numbers of rhamnose-fermenting lactobacilli, than are those whose saliva does not. Furthermore, regardless of numbers, those with rhamnose-fermenting lactobacilli are more likely to develop caries than those with rhamnose nonfermenters. The difference between 48 and 30 per cent is statistically significant.

TABLE I

TYPE I	TYPE II	TYPE III
Salicin +	Salicin +	Salicin –
Rhamnose +	Rhamnose –	Rhamnose –
Mannitol +	Mannitol +	Mannitol –
Raffinose – usually	Raffinose – usually	Raffinose + usually

In the Boulder group (fluoride-free water) there are no such differences observable. Approximately 90 per cent of those who had had no caries developed one or more lesions during the year. Eighty per cent[1] of the whole group developed new lesions. Apparently, this group is so susceptible that,

TABLE II

CHILDREN WITH NO CARIES IN 1951

COUNTS AND FERMENTATIVE TYPES (1951)	TOTAL NUMBER	NUMBER WITH INCREASED CARIES (1952)	PER CENT
Denver – 1 ppm F in drinking water			
rh + over 10,000	21	13	62
rh + (all)	50	24	48
rh –	76	23	30
Boulder – no F in drinking water			
rh + over 10,000	21	19	90
rh + (all)	27	24	89
rh –	40	36	90

TABLE III

COUNTS AND TYPES OF LACTOBACILLI DETERMINED IN 1951 CORRELATED WITH INCREASED CARIES IN 1952

FLUORIDE CONCENTRATION	TYPE I			TYPES II AND III			ALL TYPES		
	UNDER 10,000	OVER 10,000	ALL COUNTS	UNDER 10,000	OVER 10,000	ALL COUNTS	UNDER 10,000	OVER 10,000	ALL COUNTS
0 ppm									
New DS	17	247	264	90	189	279	107	436	543
No. examined	12	49	61	21	51	72	33	100	133
Mean	1.4	5.0	4.3	4.3	3.7	3.9	3.4	4.3	4.1
1 ppm									
New DS	24	65	89	25	34	59	49	99	148
No. examined	28	35	63	37	37	74	65	72	137
Mean	.8	1.9	1.4	.7	.9	.8	.75	1.3	1.1
Totals									
New DS	41	312	353	115	223	338	156	535	691
No. examined	40	84	124	58	88	146	98	172	270
Mean	1.0	3.7	2.8	2.0	2.5	2.3	1.9	3.1	2.5

DS, decayed surfaces.

during this period (with no fluoride to inhibit caries), either small numbers or large numbers of any type of lactobacilli are capable of initiating caries.

Correlation Between Caries Activity and All Factors Considered in This Study.—In Table III the relation of the number of new decayed surfaces (DS) to counts, types, and fluoride-content of water is shown. This was done in a previous paper with the extent of caries at the time of the isolation of the lactobacilli.[1] The average number of DS per child in the Boulder area (4.1) is considerably higher than in the Denver area (1.1). The average number in all those examined is also higher in those with 10,000 or more lactobacilli per milliliter of saliva (3.1) than in those with less than this number (1.9).

In the fluoride-free group the average number of DS in those with high counts and Type I lactobacilli is 5 compared with 3.7 in the group with high counts and Type II or III lactobacilli. This is similar to our previous findings relating total unfilled decayed surfaces with counts and types determined at the same time that the dental examination was made. However, the reverse is shown in the low count group. We are unable to explain this unless it is due to the small number in the group, i.e., only 12. In the Denver children the average DS is definitely higher in the high count-Type I group (1.9) than in the high-count Types II and III group (.9). It is only slightly higher in the low count-Type I group. The average DS of all those having Type I as the predominating lactobacillus in their saliva is slightly higher (2.8) than those with Type II or III (2.3). The differences pointed out here are not statistically significant, however.

TABLE IV

COUNTS AND TYPES OF LACTOBACILLI DETERMINED IN 1951 CORRELATED WITH PERCENTAGE OF NEW DECAY FOUND IN 1952

FLUORIDE CONCENTRATION	TYPE I			TYPES II AND III			ALL TYPES		
	UNDER 10,000	OVER 10,000	ALL COUNTS	UNDER 10,000	OVER 10,000	ALL COUNTS	UNDER 10,000	OVER 10,000	ALL COUNTS
0 ppm									
No. with decay	7	43	50	19	46	65	26	89	115
No. examined	12	49	61	21	51	72	33	100	133
Per cent with new decay	58	87	80	90	90	90	90	89	89
1 ppm									
No. with decay	9	21	30	10	13	23	19	34	53
No. examined	28	35	63	37	37	74	65	72	137
Per cent with new decay	32	60	46	27	35	31	29	47	38
Totals									
No. with decay	16	64	80	29	59	88	45	123	168
No. examined	40	84	124	58	88	146	98	172	270
Per cent with new decay	40	76	64	50	67	60	46	71	61

In Table IV the percentage of those with new decay is shown. In the fluoride-free area there is a greater percentage with new cavities in the low count-Type II group than in the low count-Type I group. There are no differences of significance in other groups. As pointed out previously, 90 per cent of this group had new carious lesions and the effect of both type and

numbers seems to be lost. In the fluoride area a significant difference is seen in the 60 per cent with decay in the high count-Type I group, compared to 35 per cent with decay in the high count-Types II and III group. It appears that when fluoride is present in 1 ppm concentration, a child with a large number of Type I lactobacilli is more likely to have an increase in carious lesions in the ensuing year than one who has a small number of any kind, or a large number other than Type I. Where rampant caries prevails, as in the Boulder area, an increase is likely to be seen, regardless of the number and type of lactobacillus.

Comparison of Previous Lactobacillus Counts and Types With Those Determined One Year Later.—Forty-one per cent of 136 children, whose saliva was examined and lactobacilli were isolated, one year after the first examination had a different type of lactobacillus predominating than was first found. There was an increase in the number of children from the fluoride area who had Type I predominating, but not in the nonfluoride area. This would seem to indicate that 1 ppm fluoride in the drinking water does not have an inhibiting effect on the growth or the ability of Type I lactobacillus to establish itself in the saliva. Thirty-eight per cent of those examined the second year had different lactobacillus counts when classified into those with 10,000 or more or under 10,000 lactobacilli per milliliter. As might be expected, the flora varies from year to year, both quantitatively and qualitatively, in a given individual's saliva.

Finally, as shown in Table V, there is a smaller percentage of those with 0 lactobacillus counts in the Boulder group where there was a greater increase in caries during the year than in the Denver group. There is also a smaller percentage with counts between 50 and 9,999, and a larger percentage with counts of 10,000 or more in the Boulder group. This shows a direct relationship between counts and increased caries in the two groups. It is similar to the findings relating counts with caries in the same groups examined one year previously.

TABLE V

PERCENTAGE OF CHILDREN HAVING NEGATIVE, LOW AND HIGH LACTOBACILLUS COUNTS IN DENVER AND BOULDER

			NUMBER OF LACTOBACILLI PER ML. OF SALIVA					
			0		50 TO 9,999		10,000 OR MORE	
		TOTAL NUMBER	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Boulder	1951	70	8	11	14	16	48	68
	1952	188	31	16	32	17	125	67
Denver	1951	73	12	16	33	45	28	39
	1952	196	31	16	69	35	96	49

SUMMARY

Suggestive evidence has been presented that when children whose drinking water contained 1 ppm fluoride had large numbers of rhamnose-fermenting lactobacilli in their saliva, they were more likely to have increased caries the year following this examination. When there was no fluoride in the water,

nearly all of the children had increased caries, regardless of numbers and types of lactobacilli.

The finding of large numbers of lactobacilli in the saliva, of any type, was usually associated with a rise in the number of decayed surfaces. Bacterial flora of the saliva of this age group varied considerably from one year to the next, both qualitatively and quantitatively. Fluoride in the drinking water in a concentration of 1 ppm appeared to have no effect on the type of lactobacillus which may become established.

REFERENCE

1. Clapper, W. E., Downs, R. A., and Heatherman, M. E: The Relation of Caries Activity to Lactobacillus Counts and Types and to the Fluoride Content of Drinking Water, *J. D. Res.* 32: 27, 1953.

Notice

The Thirty-second General Meeting of the International Association for Dental Research will be held at French Lick, Ind., March 19, 20, and 21, 1954.